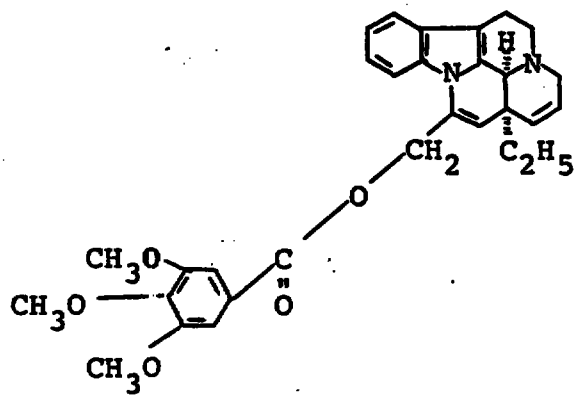
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17,18-Dehydro-Apovincaminol Derivatives

This invention relates to polycyclic compounds containing a double bond in the D-ring, a process for their preparation and pharmaceutical compositions containing them.

More particularly, the invention provides 17, 18-dehydro-apovincaminol-3',4',5'-trimethoxy-benzoate of the formula (I):

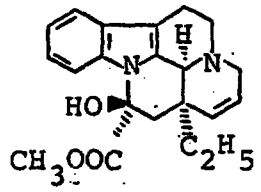


(I)

and acid addition salts thereof. According to another aspect of the invention there is provided a method for preparing 17,18-dehydro-apovincaminol-3',4',5'-trimethoxy-benzoate and acid addition salts thereof. Still another aspect of the invention is a pharmaceutical composition which comprises as an active ingredient a pharmaceutically effective amount of the compound of formula (I) or a pharmaceutically acceptable acid addition salt thereof, with at least one pharmaceutically inert carrier or diluent and optionally further pharmaceutically active substances. 17,18-Dehydro-apovincaminol is described and claimed in our Divisional Application No. 83 10731 (Ser. 2125788)

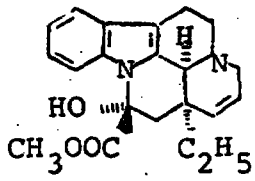
According to the invention the new 17,18-dehydro-apovincaminol-3',4',5'-trimethoxy-benzoate of formula (I)

is prepared starting from 17,18-dehydro-vincamine of formula (IVa)



(IVa)

or 17, 18-dehydro-epivincamine of formula (IVb)



(IVb)

or a mixture of the compounds of formulae (IVa) and (IVb).

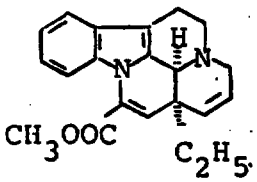
The starting compounds of formulae (IVa) and (IVb) are known in the art. It was first suggested by Wenkert [J. Am. Chem. Soc. 87(7), 1580 (1965)] that aspidosperma alkaloids can be converted into vincamine alkaloids. As a typical example the transformation vincadifformine → vincamone → vincamine was discussed.

The hypothesis was proved by Kuthey et al. [J. Am. Chem. Soc. 93(1), 255 (1971)] and was extended to the transformation tabersonine → 17,18-dehydro-vincamine. A synthesis based on the latter conversion

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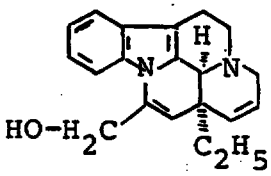
is disclosed in Belgian Patent Specification No. 818,144.

The known starting compounds have a vascotropin activity. The compound according to the invention can be prepared by treating a compound of formula (IVa) and/or (IVb) with a suitable dehydrating agent, reducing the obtained compound of formula (III):



(III)

with a selective reducing agent, preferably a complex metal hydride, and then reacting the resulting compound of formula (II):



(II)

with 3,4,5-trimethoxy-benzoic acid or a derivative thereof capable of acylation and, if desired, converting the compound of formula (I) obtained into an acid addition salt thereof.

The new compound of formula (I) and acid addition salts thereof inhibit phosphodiesterase enzyme activity and are particularly suitable for treating skin diseases accompanied by pathological cell proliferation. The

compounds also have a prophylactic effect.

Skin diseases accompanied by pathological proliferation of the epidermis are relatively frequent and involve several per cent of the population. Diseases of this kind include benign and malignant diseases, such as psoriasis atopias dermatitis, primary contact dermatitis, allergic contact dermatitis, baso- and spinocellulary carcinoma, ichthyosis, premalignus hyperkeratosis, light-induced keratosis, acne and seborrheic dermatitis. Certain diseases are only found in human beings while others may be observed in animals as well.

Since some of the skin diseases accompanied by pathological cell proliferation do not occur in animals, e.g. psoriasis, the anti-psoriatic activity of the compounds can only be demonstrated indirectly in animal tests.

Voorhees et al. [Arch. Derm. 104, 359-365 (1971)] established that the pathological proliferation is accompanied by a decrease in the level of cyclic adenosine monophosphate (c-AMP). As is well known, c-AMP is produced by adenyl cyclase and is decomposed by phosphodiesterase. Voorhees successfully influenced psoriasis by agents stimulating the activity of adenyl cyclase (e.g. norepinephrine) or inhibiting the activity of phosphodiesterase (e.g. papaverine).

When planning our model experiments we started

(14)

from the assumption that the converse of Voorhees's statement is also true, i.e. if a compound inhibits the activity of phosphodiesterase, this indirectly renders it probable that the compound is suitable for treating psoriasis or other skin diseases accompanied by pathological cell proliferation. This assumption has been verified, i.e. the compounds showing in vitro a phosphodiesterase inhibitory activity have proved effective in clinical treatment of psoriasis.

The model experiments were performed using phosphodiesterase isolated from animal tissues (rat brain, cattle brain or cattle heart). The enzyme was isolated by the technique of J. Schröder and H.V. Richenberg [Biochem. Biophys. Acta 302, 50 (1973)], whereupon the phosphodiesterase isolated was purified according to the method of J. G. Hardman and E. W. Sutherland [J. Biol. Chem. 240, 3704 (1965)] and the activity of the purified enzyme was measured by a radioisotopic method developed by G. Poch, in the presence of an excess amount of c-AMP tritium labeled (10.1 mmoles of a c-AMP substrate containing 2.59 K Bq of 3_H-c-AMP), in an incubator. The measurement was first carried out without the inhibitory substance and then in the presence of 17,18-dehydro-apovincaminol-3',4',5'-trimethoxy-benzoate as an inhibitor, after an incubation time of 20 minutes [N.S. Arch. Pharmacol. 268, 272 (1971)]. From the

test compound a 1 mmole stock solution was prepared with aqueous hydrochloric acid solution and different amounts of the stock solution were added to the enzyme preparation corresponding to 5×10^{-6}, 1×10^{-5} and 5×10^{-5} mole/lit. of the test compound. A solution of papaverine used as reference compound was added to the enzyme preparation in an analogous manner.

The activity of the solutions containing 17,18-dehydro-apovincaminol-3',4',5'-trimethoxy-benzoate and papaverine was expressed as a percentage of the control (enzyme solution without any inhibitory substance, 100%). The results obtained on an enzyme isolated from rat brain are as follows:

Test compound (enzyme inhibitor)	The effect of a 5×10^{-6} mole/lit. concentration of the test compound on enzyme activity as % of the control	1×10^{-5}	5×10^{-5}
17,18-dehydro-apovincaminol-3',4',5'-trimethoxy-benzoate.HCl	84.7	40.7	38.6
papervine.HCl	91.2	89.7	60.5

The tests on an enzyme isolated from cattle brain and cattle heart, respectively were carried out in an analogous way. On the basis of the results obtained, the enzyme activity was plotted against the logarithm of the concentration of enzyme inhibitor

(16)

(μmoles), and the inhibitor concentration which resulted in a 50 % decrease of enzyme activity (I_{50}) was read off from the curve. The results obtained are shown in the following Table:

Test compound	I_{50} (μmoles) on phosphodiesterase isolated from cattle brain	cattle heart	rat brain
17,18-dehydro-apovincaminol-3',4',5'-trimethoxybenzoate.HCl	15	3	10
Papaverine.HCl	90	50	70

The results set forth in the above Table clearly show that the new compound according to the invention was 6 times as effective as papaverine used as reference substance on an enzyme isolated from cattle brain. On an enzyme isolated from cattle heart it was 18 times as effective and on an enzyme isolated from rat brain it was 7 times as effective as papaverine.

The first clinical tests were carried out with preparations for topical use, e.g. ointments, creams, solutions, tinctures, pastes, aerosols, etc. containing the new compound according to the invention as an active ingredient. More particularly, creams containing 2 %, 1 %, 0.5 %, 0.25 % and 1.1 %, respectively

of 17,18-dehydro-apovincaminol-3',4',5'-trimethoxy-benzoate were empolyed.

Clinical tests were performed on patients suffering from psoriasis. During the tests the patients did not receive any systematic treatment, e.g. immunosuppressive, citostatic or glucocorticoid treatment, for their basic disease.

Groups of 5 were examined by the so-called plaque method. One side of symmetrical skin lesions was treated by a cream containing the active ingredient in the desired concentration, while on the other side placebo was applied. The remaining psoriatic sites on the skin were subjected to other topical treatments, for example ointments containing flumethasone pivalate and salicylic acid, as active ingredient , which are widely used for the treatment of psoriasis.

The tests were started with creams having a higher active ingredient concentration and then further patients were treated with preparations containing the smallest effective active ingredient concentration. The skin was treated 2 to 3-times a day until the symptoms disappeared or were considerably improved (1 to 6 weeks).

The effectiveness was evaluated by observing three symptoms; inflammation, infiltration and desquamation (peeling). The intensity of the symptoms was qualified by scores between 0 and 3. The total

(18)

number of scores served as a measure of the reduction of symptoms. The results were evaluated by methods of mathematical statistics. During the treatment undesirable side effects were never observed.

The results are shown in the following Table.

0 = no symptoms,

1 = moderate symptoms,

2 = strong symptoms,

3 = very strong symptoms.

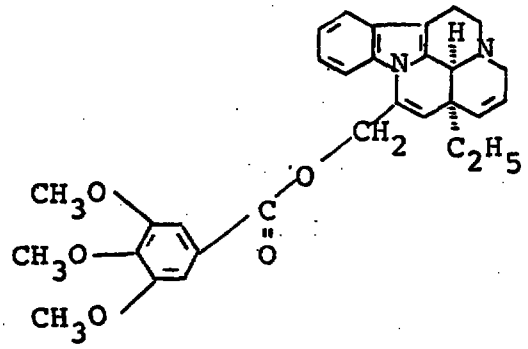
Evaluations were made before the treatment (I), after one-week treatment (II) and after a two-week treatment (III). The results set forth in the following Table are the average number of scores (total number of scores/number of patients) for creams containing 2 % of active ingredient.

	Average number of scores								
Active ingredient	Infiltration			Inflammation			Desquamation		
	I	II	III	I	II	III	I	II	III
17,18-dehydro-apovincaminol-3',4',5'-trimethoxy-benzoate	2.6	1.6	0.6	2.8	2.2	1.6	2.0	0.8	0.2

The test unambiguously proved that the compositions

can successfully be used for treating psoriasis. No side effects were observed.

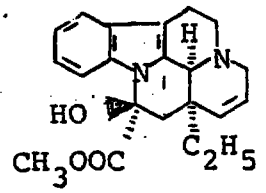
According to an aspect of the invention there is provided a process for preparing 17,18-dehydro-apo-vincaminol-3',4',5'-trimethoxy-benzoate of the formula (I):



(I)

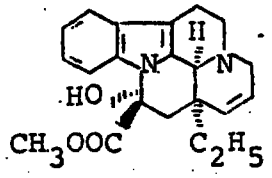
and acid addition salts thereof by

a) treating a compound of the formula (IVa):



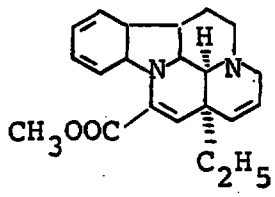
(IVa)

and/or (IVb)



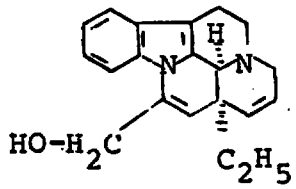
(IVb)

with a suitable dehydrating agent, reducing the resulting new compound of the formula (III):



(III)

by a selective reducing agent, preferably a complex metal hydride, and then reacting the resulting new compound of the formula (II):



(II)

with 3,4,5-trimethoxy-benzoic acid or a derivative thereof capable of acylation and, if desired, converting the compound of the formula (I) obtained into an acid addition salt thereof; or

a_2) reacting the new compound of formula (III) with a selective reducing agent, preferably a complex metal hydride, and reacting the new compound of formula (II) obtained with 3,4,5-trimethoxy-benzoic acid or a derivative thereof capable of acylation and, if desired converting the compound of formula (I) obtained into an acid addition salt thereof; or

a_3) reacting the new compound of formula (II) with 3,4,5-trimethoxy-benzoic acid or a derivative thereof capable of acylation and, if desired, converting the compound of formula (I) obtained into an acid

addition salt thereof.

According to another aspect of the invention there are provided pharmaceutical compositions having a phosphodiesterase inhibiting activity, in particular for treatment and prophylaxis of skin diseases accompanied by pathological cell proliferation, which comprises as an active ingredient a pharmaceutically effective amount of the compound of formula (I) or a pharmaceutically acceptable acid addition salt thereof, with at least one pharmaceutically inert carrier or diluent and optionally further, pharmaceutically active substances.

According to the invention the new compound of formula (I) is prepared from the known compound(s) of formula (IVa) and/or (IVb) which are treated with a dehydrating agent. As a dehydrating agent, for example sulphuric acid, a Lewis acid, formic acid, a mixture of formic acid and a lower aliphatic carboxylic acid halide, acetic anhyride or p-toluene-sulphonic acid may be employed. The reaction is preferably carried out in the presence of an organic solvent, preferably a chlorinated hydrocarbon. The compound of formula (III) obtained as a result of this reaction no longer contains a centre of asymmetry in the 14-position.

The compound of formula (III) obtained is then reacted with a selective reducing agent,

preferably a complex metal hydride. For this purpose, for example, sodium dihydro-bis(2-methoxyethoxy)-aluminate (Redal), lithium-aluminium hydride, etc. can be used. The reaction is performed in the presence of an organic solvent, preferably benzene or a homologue thereof or an ether.

The reduction results in the compound of formula (II), which is then reacted with 3,4,5-trimethoxy-benzoic acid or a derivative thereof capable of acylation.

Acylation is accomplished in the presence of an organic solvent, preferably benzene or a homologue thereof, chlorinated hydrocarbons or aliphatic ketones or pyridine. If the reaction is carried out with a 3,4,5-trimethoxy-benzoyl halide, an acid-binding agent is added to the reaction mixture in an amount equivalent ot the halogenic acid formed in the reaction or in a slight excess. As an acid-binding agent for example alkali metal carbonates, alkali metal hydrogen carbonates or organic basic amines, such as pyridine, can be employed. If the reaction is carried out with 3,4,5-trimethoxy-benzoic acid, a catalytic amount of an acid, preferably hydrochloric acid or sulphuric acid or a carboxyl activator and/or a dehydrating agent, is added to the reaction mixture. As a carboxyl activator for example halogenated phenols, preferably penta-

chlorophenol, and as a dehydrating agent for example N,N'-dicyclohexyl-carbodiimide may be employed. The acylation is carried out at a temperature between -20°C and the boiling temperature of the reaction mixture, preferably between 20°C and 60°C.

The product is isolated from the reaction mixture generally by extraction and/or evaporation.

If desired, the product obtained may be converted into an acid addition salt thereof. Preferred representatives of the inorganic acid addition salts are e.g. chlorohydrate, sulphate and phosphate salts. The preferred organic acid addition salts include the hydrogen tartrate, succinate, citrate and ascorbate salts. The salts are prepared by adding an alcoholic, ethereal or acetone solution of the acid component to the product of formula (I). The preparation of salts is carried out at a pH between 3 and 6.

The pharmaceutical compositions according to the invention generally contain 0.1 to 8.0 % by weight, preferably 0.2 to 2.0 % by weight, of active ingredient. The compositions optionally also contain further pharmaceutically active ingredients, e.g. antibiotics, citostatic agents, prostaglandins, ditranol, salicylic acid, tar, antiinflammatory agents, immunosuppressive agents, glucocorticoids and, in the case of compositions for parenteral administration, local anaesthetics. As a glucocorticoid preferably triamcinolon acetonide is

employed. The compositions are preferably provided as formulations suitable for topical, local application, e.g. creams, ointments, solutions, gelées, aerosols, aerosol foams, plasters, etc.

The active ingredient preferably is incorporated into a cream which is easy to wash off.

Creams are prepared by dissolving the active ingredient in an alcoholic solvent, preferably propylene or dipropylene glycol or a mixture thereof with water, and subsequently admixing the solution obtained with a readily smearable skin compatible fatty phase.

The fatty phase may contain cetyl, stearyl or cetostearyl alcohol, paraffin oil, glycerine mono-stearate, etc.

The creams may further contain emulsifying agents, preferably polyoxyethylene-sorbitan monooleate or monostearate and preserving agents, e.g. various benzoic acid derivatives, preferably p-hydroxy-benzoic acid methyl ester.

The creams optionally contain 0.25 to 2.0 % by weight of active ingredient, 45 to 50 % by weight of glycol, 23 to 27 % by weight of paraffin oil, 11 to 15 % by weight of stearyl alcohol and further additive(s) up to 100 %.

The active ingredient may be formulated also as an ointment which cannot be washed off with water. In this case the active ingredient is directly

incorporated into the fatty phase.

Solutions may be prepared, for example, with 20 to 40 % by weight of propylene glycol or dipropylene glycol, 40 to 55 % by weight of a 96 % ethanol and distilled water up to 100 %.

Aerosol formulations are prepared by adding a solution of the active ingredient in propylene glycol to a fat, preferably isopropyl myristate, and a suitable propellant, preferably freon.

An aerosol foam may, for example, be prepared by adding an alcoholic solution of the active ingredient to a mixture of 0.5 to 1.5 % by weight of cetostearyl alcohol, 1 to 3 % by weight of benzyl alcohol, 15 to 20 % by weight of polyoxyethylene-sorbitan monooleate or monostearate and 25 to 30 % by weight of water followed by the addition of freon.

For parenteral administration, preferably injection solutions suitable for subcutaneous or intracutaneous administration are prepared. For this purpose a salt of the active ingredient is dissolved in 0.72 % aqueous sodium chloride solution and the pH of the solution is adjusted to 5.

The products , processes and compositions according to the invention are further illustrated by the following Examples which are not intended to limit the scope of the invention in any way.

Example 1

17,18-Dehydro-apovincamine

13.4 g (38 mmoles) of a 1:1 mixture of 17,18-dehydro-vincamine and 17,18-dehydro-14-epivincamine is dissolved in 200 ml. of dry chloroform. To the solution 20 g. of dry formic acid and 11.3 g of acetyl chloride are added and the reaction mixture is allowed to stand for 2 hours. The mixture is then diluted with 200 ml. of chloroform whereupon it is shaken with 550 ml. of ice-cooled 1 N aqueous sodium hydroxide solution and subsequently 100 ml. of water. The organic phase is dried on sodium sulphate, filtered and the filtrate is evaporated to dryness. 11.9g (35.6 mmoles) of the title compound are obtained, melting at 110 to 112°C after recrystallisation from ethanol. $[\alpha]_D^{20} = +143°$ (c = 1, chloroform)

Formula: $C_{21}H_{22}O_2N_2$ (molecular weight: 334)

The same procedure is followed and the end product is obtained practically with the same yield if pure 17,18-dehydro-vincamine or the 14-epimer thereof is employed as a starting compound.

17,18-Dehydro-apovincaminol

A solution of 5.5 g (16.5 mmols) of 17,18-dehydro-apovincamine in 200 ml. of absolute diethyl ether is added to 5 g. of lithium-aluminium hydride

in 300 ml. of absolute diethyl ether dropwise, whereupon the reaction mixture is boiled for one and a half hours. The excess of the reducing agent is then decomposed with water carefully, the mixture is shaken with water, the ethereal phase is dried on sodium sulphate, filtered and the filtrate is evaporated. The evaporation residue is recrystallised from 25 ml. of ethanol to yield 4.8 g. (95 %) of the title compound, melting at 156 to 166°C.

$[\alpha]_D^{20}$ = - 26° (c = 1, chloroform)

Formula: $C_{20}H_{22}ON_2$ (molecular weight: 306)

17,18-Dehydro-apovincaminol-3',4',5'-trimethoxy-benzoate

To a solution of 10 g (32.7 mmoles) of 17,18-dehydro-apovincaminol in 200 ml. of absolute benzene 12 ml. of absolute pyridine and 11.2 g. (48 mmoles) of 3,4,5-trimethoxy-benzoyl chloride are added. The reaction mixture is kept at 40°C for one and a half hours, and is then diluted with 200 ml. of benzene. The reaction mixture is shaken with 120 ml. of ice-cooled, 1 N aqueous sodium hydroxide solution and subsequently with 50 ml. of water, the organic phase is dried on sodium sulphate, filtered and the filtrate is decolourised by passing though a column filled with alumina. By evaporation of the decolourised solution 13.2 g. (80 %) of the title compound are obtained, melting at 138 to 140°C after recrystallisation from

ethanol.

$[\alpha]_D^{20}$ = +31.5° (c = 1, chloroform)

Formula: $C_{30}H_{32}O_5N_2$ (M=500)

Example 2

17,18-Dehydro-apovincaminol-3',4',5'-trimethoxy-benzoate hydrogen tartrate

The product of Example 1 is dissolved in ethanol and a solution of D-tartaric acid is added until the precipitation of salt is complete.

The title compound is obtained, melting at 110 to 112°C (decomp.).

Analysis for $C_{34}H_{38}O_{11}N_2$ (molecular weight: 650):

calculated: N 4.3 %;

found: N 4.24 %.

u.v. spectrum$_{max}$: 209, 251, 304, 315 nm.

Example 3

The preparation of creams

17,18-dehydro-apovincaminol-3',4',5'-trimethoxy-benzoate	2 g.
propylene glycol	50 g.
paraffin oil	26 g.
polyethylene glycol 400	5 g.
stearyl alcohol	15 g.
glycerol monostearate	2 g.

The active ingredient is dissolved in propylene

glycol on a water bath, the temperature of which does not exceed 50°C. The other components are heated until molten, then cooled to 40 to 45°C with stirring. To the melt the solution of active ingredient is added with stirring and the cream obtained is stirred until it is cooled down.

Creams containing 0.25 %, 0.5, 1.0 and 1.5 % of active ingredient can be prepared in an analogous way.

Example 4

17,18-dehydro-apovincaminol-3',4',5-trimethoxy-benzoate	1.0 g.
triamcinolon acetonide	0.1 g.
propylene glycol	50.9 g.
paraffin oil	26.0 g.
polyethylene glycol 400	5.0 g.
stearyl alcohol	15.0 g.
glycerol monostearate	2.0 g.

The procedure described in Example 3 is followed except that two active ingredients are dissolved in propylene glycol.

Example 5

17,18-dehydro-apovincaminol-3',4',5'-trimethoxy benzoate	0.25 %
triamcinolon acetonide	0.1 %

propylene glycol	30.0 %
96 % ethanol	ad 100.0 %

A tincture is prepared by mixing the ingredients listed above.

Example 6

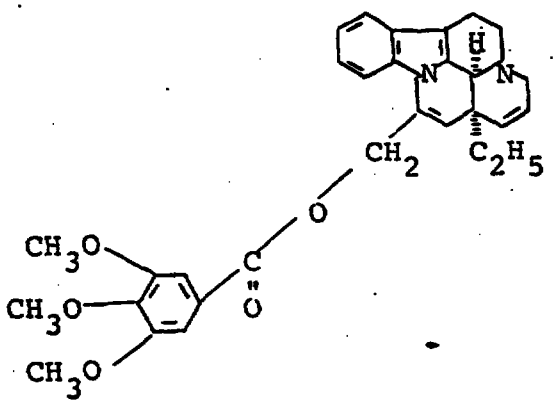
17,18-dehydro-apovincaminol-3',4',5'-trimethoxy benzoate	0.25 %
cetostearyl alcohol	1.0 %
benzyl alcohol	2.0 %
polyoxyethylene sorbitan monostearate	15.0 %
96 % ethanol	30.0 %
freon (RTM)	20.0 %
distilled water	ad 100.0 %

An aerosol foam is prepared using the above ingredients.

CLAIMS

1. 17,18-Dehydro-apovincaminol-3',4',5'-trimethoxy-benzoate or an acid addition salt thereof.

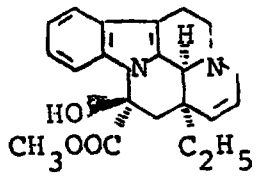
2. A process for the preparation of 17,18-dehydro-apovincaminol-3',4',5'-trimethoxy-benzoate of the formula (I):



(I)

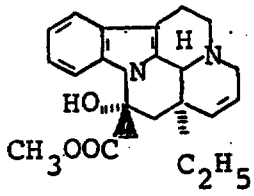
or an acid addition salt thereof, which comprises

a_1) treating a compound of the formula (IVa):



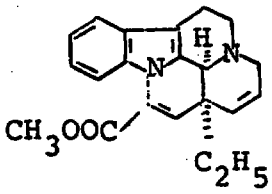
(IVa)

and/or (IVb)



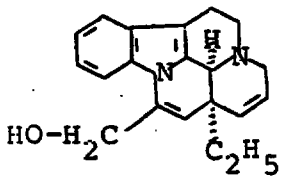
(IVb)

with a dehydrating agent, reducing the resulting compound of the formula (III):



(III)

with a selective reducing agent and reacting the resulting compound of formula (II):



(II)

with 3,4,5-trimethoxy-benzoic acid or a derivative thereof capable of acylation, and if desired converting the compound of formula (I) obtained into an acid addition salt thereof; or

a_2) reacting the compound of formula (III) with a selective reducing agent and treating the compound of formula (II) obtained with 3,4,5-trimethoxy-benzoic acid or a derivative thereof capable of acylation, and if

desired converting the compound of formula (I) obtained into an acid addition salt thereof; or

a_3) reacting the compound of formula (II) with 3,4,5-trimethoxy-benzoic acid or a derivative thereof capable of acylation, and if desired converting the compound of formula (I) obtained into an acid addition salt thereof.

3. A process as claimed in Claim 2, wherein the selective reducing agent is a complex metal hydride.

4. A process as claimed in claim 2, or 3, which comprises using formic acid as a dehydrating agent in process variant a_1).

5. A process as claimed in any of claims 2 to 4, which comprises using lithium-aluminium hydride as a selective reducing agent in process variant a_1) or a_2).

6. A process as claimed in any of claims 2 to 5, which comprises using 3,4,5-trimethoxybenzoyl chloride as an acylating agent in process variant a_1), a_2) or a_3).

7. A process as claimed in any one of claims 2 to 6, which comprises carrying out the reactions in an organic solvent.

* C L M S *

8. A process for the preparation of a pharmaceutical composition, which comprises admixing 17,18-dehydro-apovincaminol-3',4',5'-trimethoxy-benzoate or a pharmaceutically acceptable acid addition salt thereof with at least one pharmaceutically inert carrier or diluent and optionally at least one further pharmaceutically active substance.

9. A pharmaceutical composition for treatment and prophylaxis of diseases accompanied by pathological cell proliferation, which comprises as an active ingredient a pharmaceutically effective amount of 17,18-dehydro-apovincaminol-3',4',5'-trimethoxy-benzoate or an acid addition salt thereof in combination with at least one pharmaceutically inert carrier or diluent and optionally at least one further pharmaceutically active ingredient.

10. A pharmaceutical composition as claimed in claim 9, which comprises 0.1 to 8.0 % by weight of 17,18-dehydro-apovincaminol-3',4',5'-trimethoxy-benzoate or an acid addition salt thereof.

11. A pharmaceutical composition as claimed in claim 9, comprising 0.2 to 2.0 % by weight of 17,18-dehydro-apovincaminol-3',4',5'-trimethoxy-benzoate or acid addition salt thereof.

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12. A compound as claimed in claim 1, substantially as hereinbefore described with reference to any of the Examples.

13. A process as claimed in claim 2, substantially as hereinbefore described with reference to Example 1 or 2.

14. A compound as claimed in claim 1, prepared by a process as claimed in any of claims 2 to 7 and 13.

15. A pharmaceutical composition as claimed in claim 9,

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substantially as hereinbefore described with reference to any of Examples 3 to 6.

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