

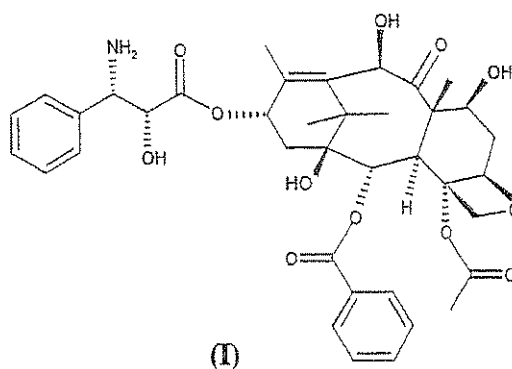
SEMISYNTHESIS PROCESS FOR THE PREPARATION OF 10-DEACETYL-N-DEBENZOYL-PACLITAXEL

Field of the invention

Object of the present invention is a new semisynthesis process for the preparation of 10-deacetyl-N-debenzoyl-paclitaxel (I), a useful synthon for the preparation of taxanes with anti-tumour activity.

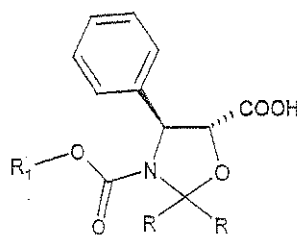
5 The invention also relates to a process for the preparation of 10-deacetyl-bis-7,10-trichloroacetylbaccatin III with a content of the corresponding 7- or 10 mono-trichloroacetyl derivatives lower than 0.1% as determined by HPLC.

The invention also concerns a process for the preparation of Docetaxel having a purity degree higher than 99%, by subjecting the intermediate (I)
10 obtained by the process of the invention to reaction with di-*tert*-butyl dicarbonate as well as pharmaceutical compositions comprising said high-purity Docetaxel.

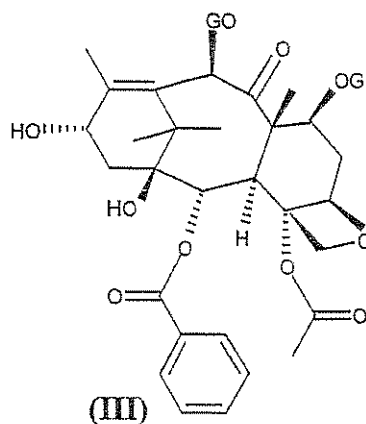


State of the art

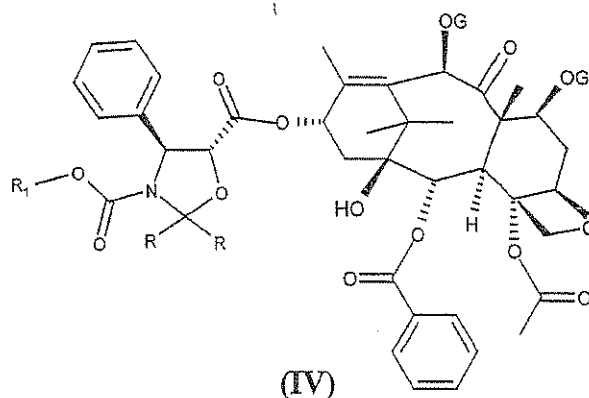
15 A process comprising the esterification with oxazolidines of formula (II)



of 10-deacetylbaccatin protected at the 7- and 10-positions of formula (III)



to give esters of formula (IV)



has been disclosed in WO 94/07877 for the synthesis of synthon (I), reported in the literature in the early '90s (F. Guéritte-Voegelein et al., J. Med. Chem. 34, 992, 1991).

Liberation of the amino function at the 3'-position and hydroxy groups at the 2', 7- and 10-positions from the esters of formula (IV) affords synthon (I).

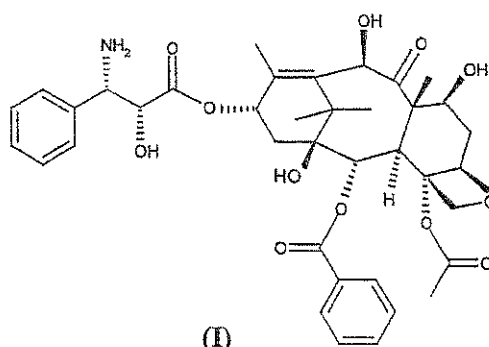
In particular, according to the above-cited patent application, groups R can be hydrogen, alkyl, alkoxy or variously-substituted phenyl and R₁ is alkyl substituted with one or more chlorine atoms. Groups G are alkylsilyl or R₁-O-CO- groups wherein R₁ is as defined above.

Starting from the intermediates of formula (IV), the hydroxy and the amino functions are liberated by reduction with zinc and acids and, when groups G are alkylsilyl, the hydroxy functions are liberated by acid treatment,

for example with hydrofluoric acid.

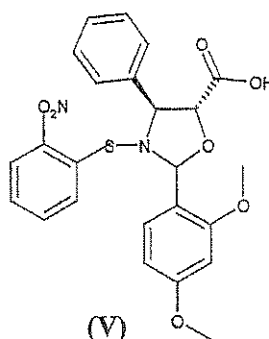
Summary of the invention

The present invention, in a first embodiment thereof, concerns a process for the preparation of 10-deacetyl-N-debenzoyl-paclitaxel (I)

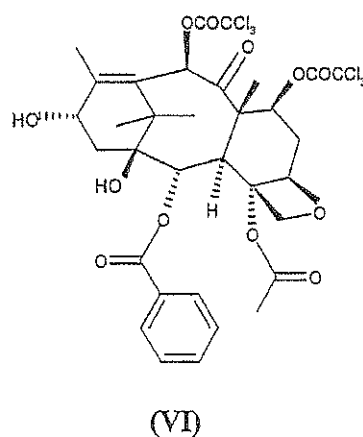


comprising the following steps:

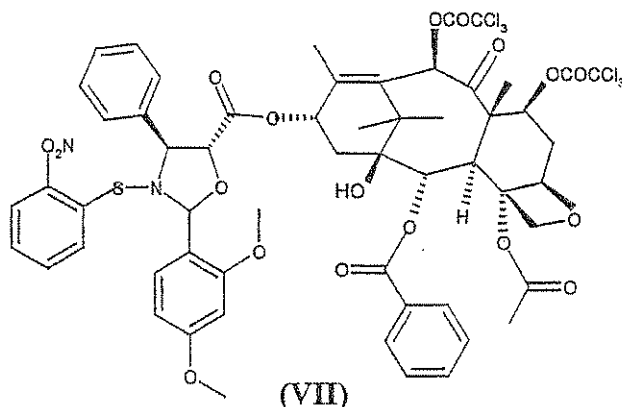
- a) reaction of 2-(2,4-dimethoxyphenyl)-3-(2-nitrobenzenesulfenyl)-4(*S*)-phenyl-5(*R*) - oxazolidinecarboxylic acid (V)



with 10-deacetyl-bis-7,10-trichloroacetylpaclitatin III (VI)

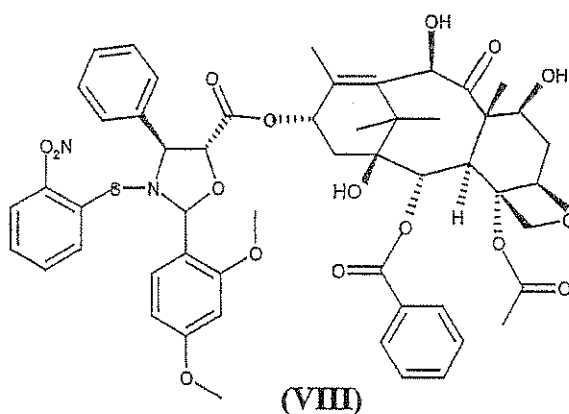


to give 2-(2,4-dimethoxyphenyl)-3-(2-nitrobenzensulfenyl)-4(*S*)-phenyl-5(*R*)-oxazolidine carboxylic acid, 10-deacetyl-7,10-bis-trichloroacetylpaclitaxin III 13-yl-ester (VII)



5

- b) hydrolysis of the trichloroacetyl groups at the 7- and 10- positions of the compound of formula (VII) to give 2-(2,4-dimethoxyphenyl)-3-(2-nitrobenzensulfenyl)-4(*S*)-phenyl-5(*R*)-oxazolidine carboxylic acid, 10-deacetylpaclitaxin III 13-yl-ester (VIII)



10

- c) acid treatment of the compound formula (VIII) to give 10-deacetyl-N-debenzoyl-paclitaxel (I).

The invention also provides as novel intermediates 2-(2,4-Dimethoxyphenyl)-3-(2-nitrobenzensulfenyl)-4(*S*)-phenyl-5(*R*)-oxazolidine carboxylic acid, 10-deacetyl-7,10-bis-trichloroacetylpaclitaxin III 13-yl-ester (VII) and 2-(2,4-Dimethoxyphenyl)-3-(2-nitrobenzensulfenyl)-4(*S*)-phenyl-5(*R*)-oxazolidine carboxylic acid, 10-deacetylpaclitaxin III 13-yl-ester (VIII).

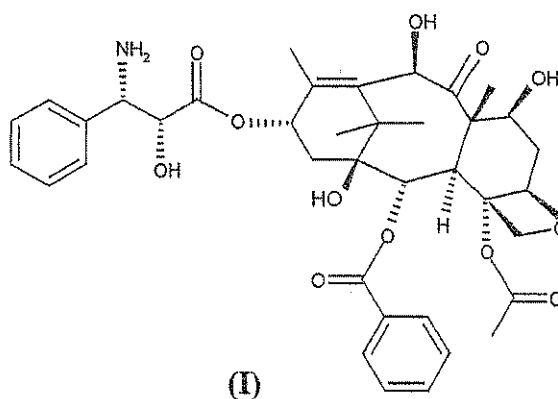
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The invention also concerns a process for the preparation of 10-deacetyl-bis-7,10-trichloroacetylbaccatin III with a content of the corresponding 7- or 10 mono-trichloroacetyl derivatives lower than 0.1% as determined by HPLC, comprising the silica gel chromatography of the reaction mixture.

5 A further object of the invention is provided by Docetaxel having a purity degree higher than 99% as well as pharmaceutical compositions comprising it.

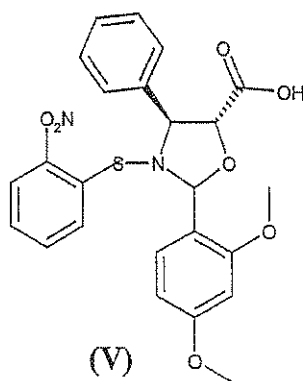
Description of the invention

10 The present invention relates to a process for the synthesis of synthon (I)



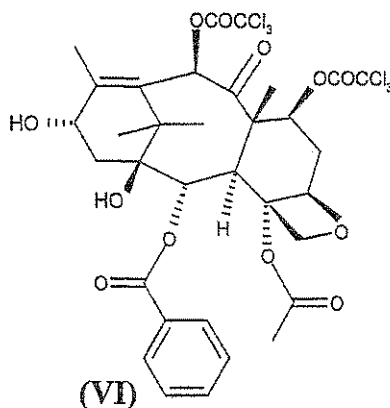
in high yield and/or quality. The process moreover does not require the polluting or difficult to handle reagents, such as zinc and hydrofluoric acid.

15 The process consists in the reaction of 2-(2,4-dimethoxyphenyl)-3-(2-nitrobenzenesulfenyl)-4(*S*)-phenyl-5(*R*)-oxazolidinecarboxylic acid (V)

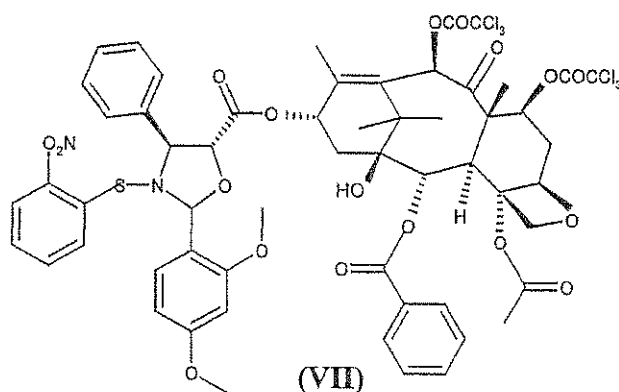


with 10-deacetyl-bis-7,10-trichloroacetylbaccatin III (VI)

6



to give the ester (VII)



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wherefrom synthon (I) is obtained after liberation of the amino and hydroxy functions.

The compound of formula (VII) is novel and is a further object of the present invention.

10 The oxazolidine acid (V) either 2R, 2S or a mixture thereof, is equally useful in the synthesis, since the chiral center at the 2-position of the oxazoline ring is removed from intermediate (VII) upon liberation of the hydroxy and amino functions. In other words, the relative ratio between the diastereoisomers does not impair the performance of the synthesis.

15 The oxazolidine acid (V) is easily prepared by acid treatment of the corresponding alkali salts, whose preparation has been disclosed in WO 03/087077 A1.

Compared to other oxazolidine acids, acid (V) is characterised by

remarkable stability; which allows to easily carry out the esterification with synthon (VI).

Moreover, after the esterification, the liberation of the amino and hydroxy functions contained in the acid residue can be easily carried out by treatment with acids, without the need to adopt drastic conditions.

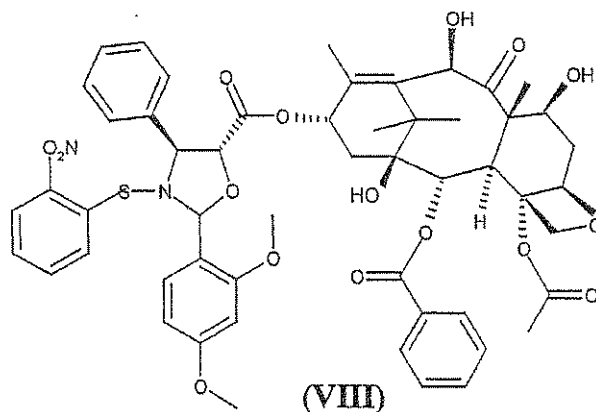
The taxane synthon (VI) can be obtained from the natural metabolite 10-deacetylbaccatin III through esterification of the 7- and 10-positions by treatment with trichloroacetic acid activated derivatives, according to known esterification methods. Preferably, synthon (VI) is obtained by reaction with trichloroacetic acid chloride at a temperature around 0°C, using pyridine as the solvent. Preferably, 10-deacetyl-bis-7,10-trichloroacetylbaccatin III (VI), is purified from its corresponding 7- and 10-mono-trichloroacetyl esters by silica gel chromatography or equivalent methods. The residual amount of said impurities should not exceed 0.1% as measured by HPLC % peaks.

According to the present invention, the esterification of (VI) with the oxazolidine acid (V) to give (VII) can be carried out in the presence of a condensing agent, such as a diimide, for example dicyclohexylcarbodiimide, and an activating agent, for example 4-dimethylamino-pyridine or 4-pyrrolidino-pyridine in a solvent selected from an ether, such as ethyl ether, diisopropyl ether, tetrahydrofuran or dioxane; an ester, such as ethyl, propyl or butyl acetate; an aromatic hydrocarbon, such as benzene, toluene or *o*-, *m*-, *p*-xylene; or a halogenated aliphatic hydrocarbon, for example methylene chloride, chloroform or dichloroethane. Carrying out the esterification in methylene chloride at the temperature of about 20°C is particularly advantageous.

The preparation of synthon (I) from ester (VII) requires removal of the trichloroacetyl groups from the 7- and 10-positions and liberation of the amino and hydroxy functions from the oxazolidine residue.

As mentioned above, the amino and hydroxy functions can be easily liberated from the oxazolidine residue by acid treatment. On the contrary, the hydrolysis of the trichloroacetic esters can be conveniently carried out by mild alkaline treatment, preferably by reaction with ammonium hydroxide.

5 It has been observed that, if the liberation of the amino and hydroxy functions from the oxazolidine residue is carried out first, massive migration of a trichloroacetyl group from the baccatin residue to the free amino function occurs, with consequent formation of a trichloroacetamido function, which could be transformed in an amino function only under conditions that would
10 be detrimental to the structure of the baccatin skeleton. As a consequence, the preparation of synthon (I) requires first the removal of the trichloroacetic groups at the 7- and 10-positions of (VII) to give ester (VIII).



Also the compound of formula (VIII) is novel and is a further object of
15 the present invention. Preferably, the removal of the trichloroacetic groups is carried out at room temperature by treatment with ammonium hydroxide in tetrahydrofuran as the solvent.

The liberation of the amino and hydroxy functions is carried out by treatment with acids, preferably with aqueous hydrochloric acid, in alcoholic
20 solution, for example in methanol at a temperature of about 20°C. After dilution with water and removal of reaction by-products with organic solvents, such as aliphatic hydrocarbons and halogenated aliphatic hydrocarbons, for

example *n*-hexane and methylene chloride, synthon (I) is isolated by alkalization of the aqueous phase, extraction in an organic solvent, for example methylene chloride or ethyl acetate, concentration and precipitation in an aliphatic hydrocarbon, such as *n*-hexane. The process of the invention
5 provides synthon (I) with purity higher than 98%, without chromatographic purifications.

Docetaxel can be advantageously obtained from said intermediate with a purity degree higher than 99%, preferably higher than 99.4%, by reaction with di-*tert*-butyl dicarbonate.

10 The reaction is preferably carried out in solvents such as alcohols (methanol, ethanol, isopropanol, preferably ethanol), chlorinated hydrocarbons (methylene chloride, chloroform, preferably methylene chloride) or mixtures thereof, in the absence of bases.

The process is advantageous since Docetaxel may be obtained in high
15 purity without cumbersome chromatographic purifications, by crystallizations from suitable solvents, preferably from ethanol/water and/or acetone/hydrocarbon mixtures. Docetaxel obtained using the process subject of the present invention is characterized by a purity degree higher than 99% (HPLC area%) and content of 7-*epi* docetaxel and 10-dehydrodocetaxel lower
20 than 0.1% each (HPLC area%).

The invention will be now illustrated in more detail in the following examples.

EXAMPLES

Example 1 - 10-Deacetyl-7,10-bistrichloroacetylbaaccatin III (VI)

25 10-Deacetylbaaccatin III (15 g) is treated with 6.6 ml of trichloroacetyl chloride in 60 ml of pyridine at 0-5°C for 1 hour under stirring. The mixture is diluted with 100 ml of methylene chloride and 100 ml of 4 N hydrochloric acid. The phases are separated and the organic one is washed with 100 ml of

4 N hydrochloric acid and 50 ml of water saturated with sodium chloride. The organic phase is concentrated under vacuum and the residue is taken up with 100 ml of toluene. Product (VI) is collected by filtration and dried under vacuum at 50°C. The latter is dissolved at 35°C in CH₂Cl₂ (80 ml) and
5 purified by column chromatography using 800 g of Kieselgel 60 Merck (eluent: CH₂Cl₂). The fractions are combined (TLC: CH₂Cl₂) and checked by HPLC. The total content of mono 7 and 10.-trichloroacetyl baccatin III must be less than 0.1%. Purified compound (VI) is precipitated in toluene to yield (17.8 g, 21.4 mmol, 660/26/B, A% purity: 99%, yields: 78%)

10 **Example 2 - 2-(2,4-Dimethoxyphenyl)-3-(2-nitrobenzensulfenyl)-4(S)-phenyl-5(R)-oxazolidine carboxylic acid, 10-deacetyl-7,10-bis-trichloroacetyl baccatin III 13-yl-ester (VII)**

A solution containing 10.3 g of (V) in the form of sodium salt in 100 ml of water is cooled to 0-5°C and adjusted to pH 2-3 with a 2 M sodium
15 bisulfate solution. The reaction mixture is stirred at 0°C for 15 minutes and then CH₂Cl₂ (70 ml) is added. The two phases are separated and the aqueous layer extracted once with CH₂Cl₂ (1x50 ml). The combined organic phases are washed with a saturated solution of NaCl (1x25 ml) (360 g/l) and dried over anhydrous MgSO₄ (3 g, KF 0.12%). After filtration, the solution is
20 concentrated under vacuum at room temperature until 100 mL. To the yellow solution 12 g of (VI) are added, followed by 0.175 g (1.42 mmol) of dimethylaminopyridine (DMAP) and, after complete dissolution of the reagent, 5.88 g of dicyclohexylcarbodiimide (DCC). The reaction mixture is stirred at room temperature for an hour. No starting (VI) is detected by TLC
25 (ethyl acetate/hexane:1/2, detection by spraying with a solution containing H₂SO₄ (31 mL), ammonium molybdate (19 g) and (NH₄)₄Ce(SO₄)₄·2 H₂O (1.9 g) in water (500 mL) and heating at 130°C for 5 min). The precipitate of dicyclohexylurea (DCU) formed is filtered off and washed with CH₂Cl₂

(1x20 mL). The chloromethylene solution is evaporated to dryness yielding 24 g of (VII).

Example 3 - 2-(2,4-Dimethoxyphenyl)-3-(2-nitrobenzensulfenyl)-4(*S*)-phenyl-5(*R*)-oxazolidine carboxylic acid, 10-deacetylbaaccatin III
5 **13-yl-ester (VIII)**

A solution containing 24 g of (VII) in 100 ml of tetrahydrofuran is concentrated under vacuum, the residue taken up with 150 ml of tetrahydrofurane (THF) and the mixture concentrated under vacuum till 100 ml.

10 Conc ammonium hydroxide 33% (NH₄OH, 1.8 ml, 30 mmol) is added at room temperature in 5 minutes and the reaction mixture is stirred at room temperature for two hours. TLC of the mixture shows no compound (VII) (ethyl acetate/hexane: 4/3). The solution is concentrated under vacuum and the residue taken up with MeOH (125 ml). The suspension is stirred for 2 hours.
15 The precipitate is filtered through a sintered glass filter and washed with (10 ml) of MeOH to get compound (VIII) (13 g, 12 mmol, HPLC A%= 93%, yield 84%). The mother liquor contains 9.3 g of residue to be discarded.

Example 4 - 10-Deacetyl-N-debenzoyl-paclitaxel (I)

A suspension of 13 g of (VIII) in 260 ml of methanol is treated for
20 30 minutes at room temperature under stirring with 4.2 ml of concentrated aqueous hydrochloric acid diluted with 130 ml methanol. The reaction mixture is stirred at room temperature for four hours and the suspension becomes a clear yellow solution. TLC of the mixture shows no compound (VIII) (ethyl acetate/hexane: 4/3). The solution is slowly diluted with water (350 ml) (to
25 avoid the formation of precipitate) and the homogeneous solution is stirred at room temperature for 30 minutes. CH₂Cl₂ (200 ml) is added, the two phases are separated and the aqueous layer extracted again with CH₂Cl₂ (2x100 ml). The organic phases are eliminated. The hydro-alcoholic phase is cooled down

to 0-5°C and diluted with CH₂Cl₂ (1x100 ml). Under vigorous stirring at 0-5°C conc. ammonia (3.1 ml, NH₄OH) is added dropwise (a 1 degree increase of the temperature is obtained) up to pH=7-8. The biphasic reaction is stirred at the same temperature for 20 minutes, then the phases are separated and the aqueous layer is extracted with CH₂Cl₂ (5x100 ml).

The combined organic layers are concentrated under vacuum till 100 ml and at room temperature under stirring the product crystallizes. The precipitate is filtered through a glass sintered filter and after drying under vacuum at 40°C overnight, 7.5 of the title compound are obtained.

10 **Example 5 - Preparation of Docetaxel**

Compound (I) (16 g, HPLC purity assay: 90.57%, 20.49 mmol) is dissolved in a mixture 1:1 of absolute EtOH and CH₂Cl₂ (320 ml) and to the slightly yellowish solution di-*tert*-butyl dicarbonate (BOC)₂O in CH₂Cl₂ (24.18 mmol, 5.27 g dissolved in 5 ml of CH₂Cl₂) is added. At the end of the addition the reaction mixture is stirred for 16 hours at room temperature. TLC shows no compound (I) (CH₂Cl₂/MeOH:9/1, detection by spraying with a solution containing H₂SO₄ (31 ml), ammonium molybdate (19 g) and (NH₄)₄Ce(SO₄)₄·2 H₂O (1.9 g) in water (500 ml) and heating at 130°C for 5 min). The CH₂Cl₂ is distilled off and acetic acid is added (0.39 ml) to the solution. The acidic ethanol solution is heated at 50°C and pure water (320 ml) is added dropwise. The mixture is left at 50°C for an hour and at room temperature for additional 2 hours. The precipitate is filtered through a glass sintered filter and transferred within a vacuum oven and maintained under vacuum at 40°C overnight to yield 16.75 g of semi-purified Docetaxel and 1 g of mother liquor that can be eliminated.

The crude product is crystallized twice: semi-purified Docetaxel is dissolved at 50°C in 95% ethanol (160 ml) and acetic acid (0.39 ml) is added. The mixture, after the addition of pure water (320 ml), is left at 50°C for an

hour and at room temperature for additional 2 hours. The precipitate is filtered through a glass sintered filter and transferred within a vacuum oven and maintained under vacuum at 40°C overnight to get 15.25 g of Docetaxel and 0.4 g of mother liquor that can be eliminated. The second crystallization is performed re-dissolving the product at 30°C in acetone (150 ml) and adding heptane (150 ml). The mixture is left at room temperature for three hours. The precipitate is filtered through a glass sintered filter and transferred within a vacuum oven and maintained under vacuum at 40°C overnight to get 13.9 g of Docetaxel (HPLC purity higher than 99.4%, <0.1% of 7-epi docetaxel and <0.1% 10-dehydrodocetaxel).

Example 6 - Alternative preparation of Docetaxel

De-BOC Docetaxel (30.0 g, 42 mmol, 98% HPLC purity, 0.2% 7-epi isomer) is loaded in 1 l reactor and then 60 ml dichloromethane, 150 ml absolute ethanol and 73 µl glacial acetic acid (3% mol) are added at 25°C to obtain a suspension.

BOC anhydride (11.0 g, 51 mmol) dissolved in 30.0 ml DCM is added dropwise to the suspension at 25°C; a clear solution is obtained at the end of the addition.

After 3 hours the reaction is stopped by quenching with glacial acetic acid (0.7 ml, 30% mol) and dichloromethane is distilled off at 30°C under vacuum. Absolute ethanol (90 ml) is then added and distilled off under the same conditions.

The clear solution is heated to 50°C and water (570 ml) is added dropwise in about 3 h. The suspension is stirred at 50°C for 1 h and then it is cooled in 1 h to 25°C and stirred at this temperature for 16 hours.

The white solid is filtered and washed twice with a solution of water (40 ml) and absolute ethanol (18 ml).

The crude material is put in a reactor with 250 ml ethanol and 630 µl

glacial acetic acid.

The mixture is heated at 50°C, complete dissolution occurred. Water (570 ml) is added dropwise in about two hours. The mixture is then cooled in 1 h to 25°C and, after 90 min, the suspension is filtered on gooch P3 and
5 washed once with a solution of water (40 ml) and absolute ethanol (18 ml).

Docetaxel, obtained as a white solid, is dried under vacuum at 55°C for 16 hours, final weight of dried solid: 32.6 g.

Crude Docetaxel (5.0 g, 6.2 mmol) is loaded in 500 ml reactor and dissolved at 50°C in acetone (50 ml).

10 *n*-Heptane (50 ml) are then slowly added at 50°C in about 1 h. The suspension thus obtained is stirred for 1 h at 50°C and then cooled to 25°C and stirred at the same temperature for 16 h.

The suspension is filtered on gooch P3, washed once with *n*-heptane (15 ml) and dried at 55°C under vacuum for 16 h, obtaining 4.40 g of
15 docetaxel as a white solid (89% yield, >99.5% purity HPLC, <0.10% of 7-epi docetaxel and <0.10% 10-dehydrodocetaxel).

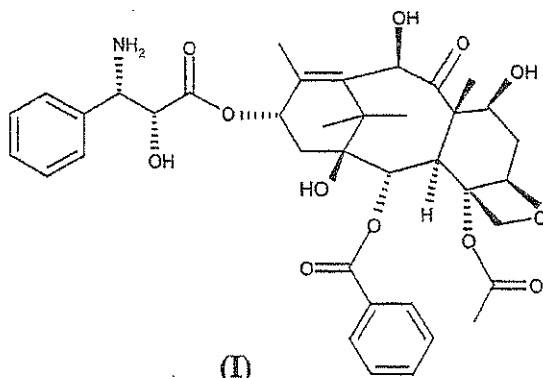
Brief description of the drawings

Figure 1. XRD diffractogram of sample prepared by procedure of example 5.

20 Figure 2. DSC thermogram of sample prepared by procedure of example 5.

CLAIMS

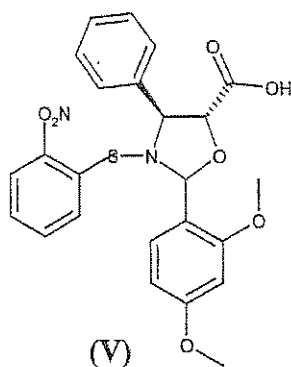
1. A process for the preparation of 10-deacetyl-N-debenzoyl-paclitaxel (I)



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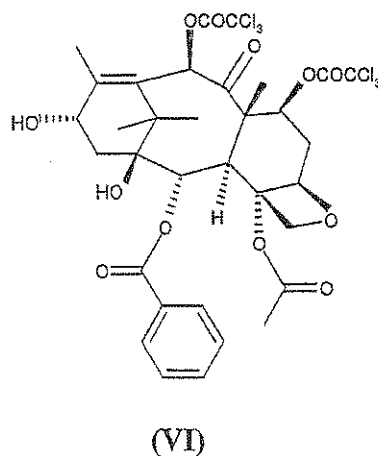
comprising the following steps:

- a) reaction of 2-(2,4-dimethoxyphenyl)-3-(2-nitrobenzenesulfenyl)-4(*S*)-phenyl-5(*R*) - oxazolidinecarboxylic acid (V)

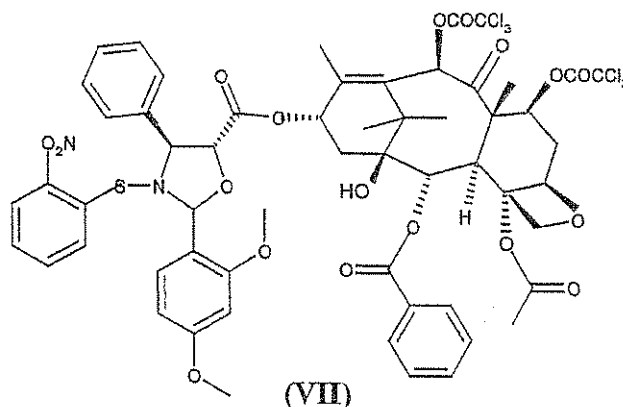


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with 10-deacetyl-bis-7,10-trichloroacetylbaaccatin III (VI)

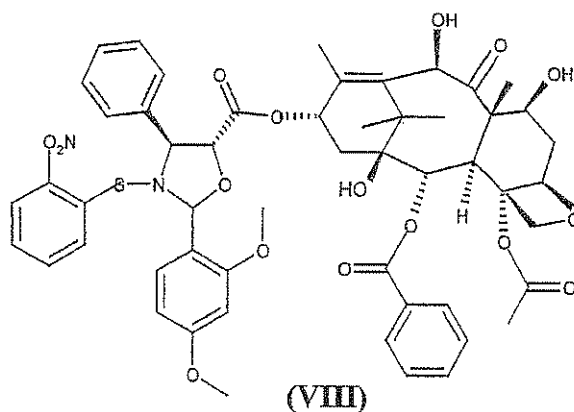


to give 2-(2,4-dimethoxyphenyl)-3-(2-nitrobenzensulfenyl)-4(*S*)-phenyl-5(*R*)-oxazolidine carboxylic acid, 10-deacetyl-7,10-bis-trichloroacetylpaclitaxin III 13-yl-ester (VII)



5

- b) hydrolysis of the trichloroacetyl groups at the 7- and 10- positions of the compound of formula (VII) to give 2-(2,4-dimethoxyphenyl)-3-(2-nitrobenzensulfenyl)-4(*S*)-phenyl-5(*R*) -oxazolidine carboxylic acid, 10-deacetylpaclitaxin III 13-yl-ester (VIII)

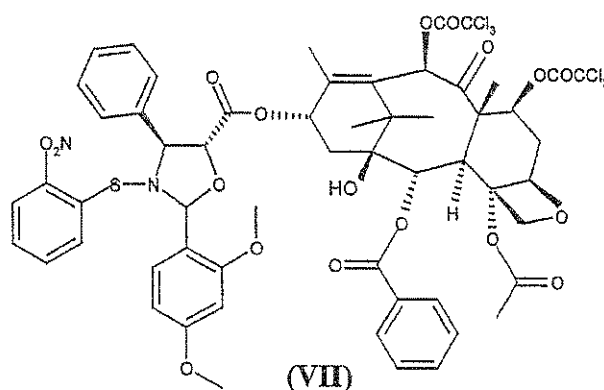


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- c) acid treatment of the compound formula (VIII) to give 10-deacetyl-N-debenzoyl-paclitaxel (I).
2. A process according to claim 1 wherein step a) is carried out in a solvent selected from an ether, an ester, an aromatic hydrocarbon or a halogenated aliphatic solvent.
3. A process according to claim 2 wherein the aliphatic halogenated hydrocarbon is methylene chloride.

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4. A process according to any one of claims 1 - 3 wherein step a) is carried out in the presence of a condensing agent and an activating agent.
5. Process according to claim 4 wherein the condensing agent is dicyclohexylcarbodiimide and the activating agent is 4-dimethylamino-pyridine.
6. A process according to any one of claims 1 to 5 wherein step b) is carried out with ammonium hydroxide in tetrahydrofuran as the solvent.
7. A process according to any one of claims from 1 to 6 wherein step c) is carried out with a methanol solution of aqueous hydrochloric acid.
- 10 8. 2-(2,4-Dimethoxyphenyl)-3-(2-nitrobenzensulfenyl)-4(*S*)-phenyl-5(*R*)-oxazolidine carboxylic acid, 10-deacetyl-7,10-bis-trichloroacetyl-13-yl-ester (VII)



- 15 9. 2-(2,4-Dimethoxyphenyl)-3-(2-nitrobenzensulfenyl)-4(*S*)-phenyl-5(*R*)-oxazolidine carboxylic acid, 10-deacetyl-7,10-bis-trichloroacetyl-13-yl-ester (VIII)

