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(54) Title: SEMI-SYNTHETIC TAXANES WITH ANTI-TUMOURAL ACTIVITY

(57) Abstract

The present invention relates to novel derivatives obtained by oxidation and the stereospecific reduction of 10-deacetylbaccatine III, and successive esterification with a variously substituted isoserine chain to prepare taxol analogues. The products of the invention have cytotoxic and anti-tumoural activities and, when suitably formulated, can be administered by injection and/or orally.

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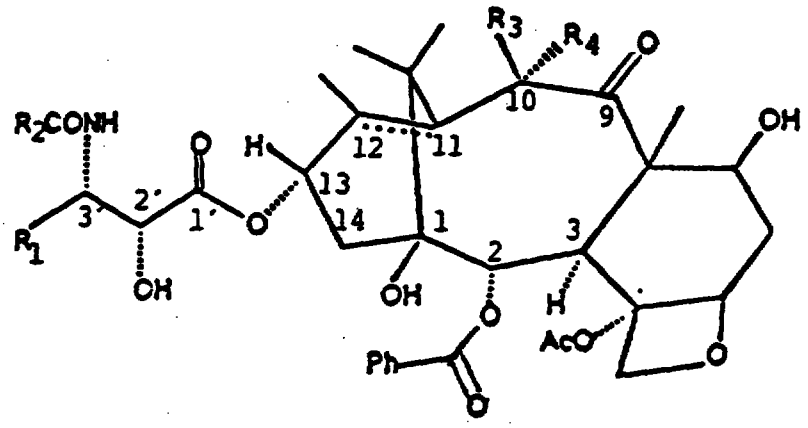
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SEMI-SYNTHETIC TAXANES WITH ANTI-TUMOURAL ACTIVITY

Diterpenes with taxane skeletons, and in particular taxol, are known to have an anti-tumoural action against numerous human tumours. However, the use of these drugs, particularly taxol, involves some drawbacks due to unwanted side effects. For this reason, and since these anti-tumoural treatments rapidly induce resistance, the development of new molecules whose use reduces the problems observed in clinical use is of interest.

The present invention relates to novel taxane-skeleton derivatives obtained by semisynthesis and having a powerful anti-tumoural activity. The derivatives of the invention have formula (1):



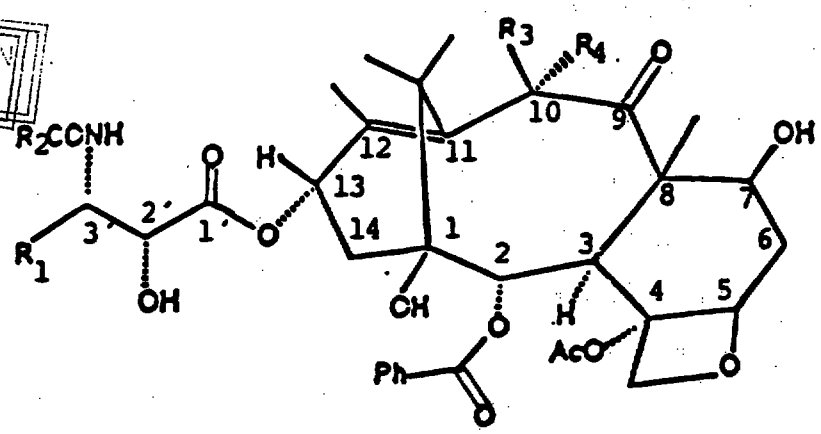
(1)

They can be divided into two series:

a) taxane derivatives containing a double olefinic bond at the 11,12- position and a hydroxy or acyloxy group at the 10α-position (taxanes of formula 1a)

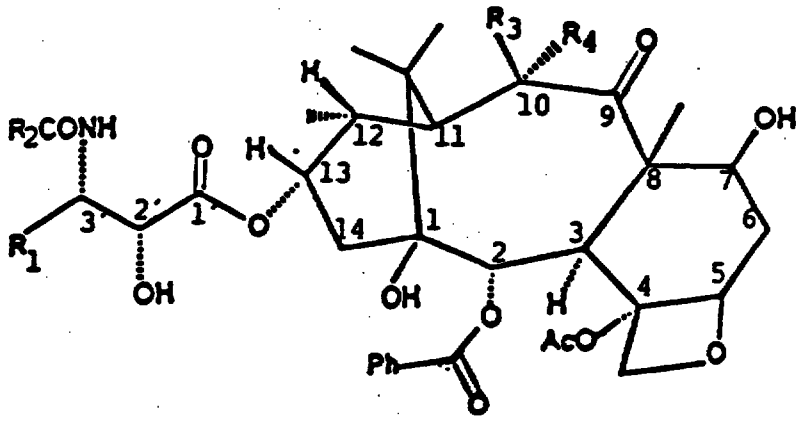
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(1a) (R_3 = H; R_4 = OH or acyloxy)

b) taxane derivatives containing a single bond between the carbon atoms in 11 and 12, the methyl in 12 being α-oriented, and a hydroxy or acyloxy group in position 10β (taxane of formula 1b)



(1b) (R_3 = OH or acyloxy; R_4 = H)

In taxanes of general formula (1), R_1 and R_2, which may be the same or different, are a C_1-C_8 alkyl, C_2-C_8 alkenyl, aryl (preferably phenyl) or heteroaryl group. R_2 can also be a tert-butoxy group.

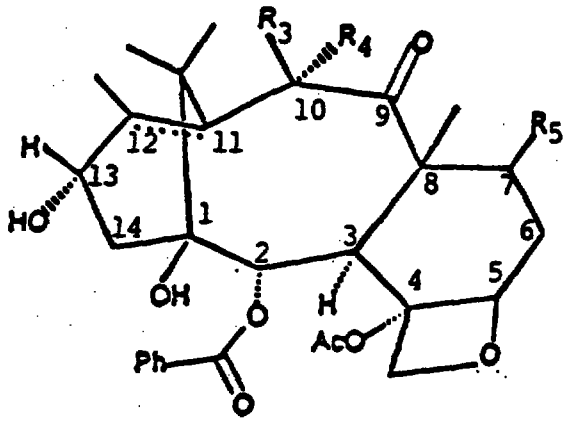
In compounds of formula (1a), R_3 is hydrogen and R_4 is a hydroxy or C_2-C_8 acyloxy group.

In compounds of formula (1b), R_3 is a hydroxy or C_2-C_8 acyloxy group and R_4 is hydrogen.

Taxanes of formula (1) are prepared by

esterificating at the 13-position the new syntones of formula (2), using suitably activated isoserine chains as acylating agents, according to what reported in literature for the semisynthesis of taxol and its analogues) (see, for example, EP-A- 400971, 1992, Fr. Dem. 86, 10400; E. Didier et al., Tetrahedron Letters 35, 2349, 1994; E. Didier at al., ibid. 35, 3063, 1994).

In formula (2)



(2)

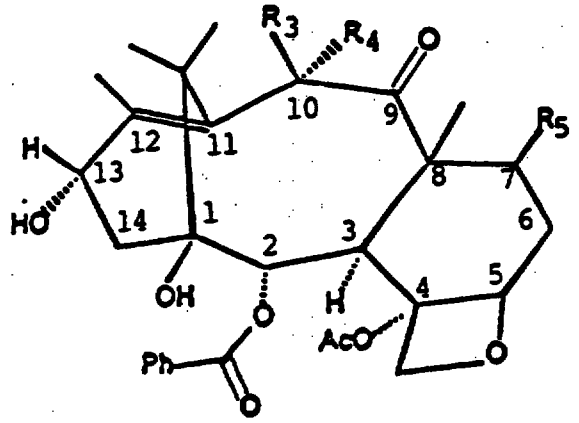
wherein:

when a double olefinic bond is present at the 11,12-position, R_3 is a hydrogen atom, R_4 and R_5 are hydroxy, C_2-C_8 acyloxy, alkylsilyloxy or 2,2,2-trichloroethoxy-carbonyloxy groups;

when a double olefinic bond is not present at the 11,12- position, the methyl at the 12- position is α-oriented, R_4 is a hydrogen atom, R_3 and R_5 are hydroxy, C_2-C_8 acyloxy, alkylsilyloxy or 2,2,2-trichloroethoxy-carbonyloxy groups.

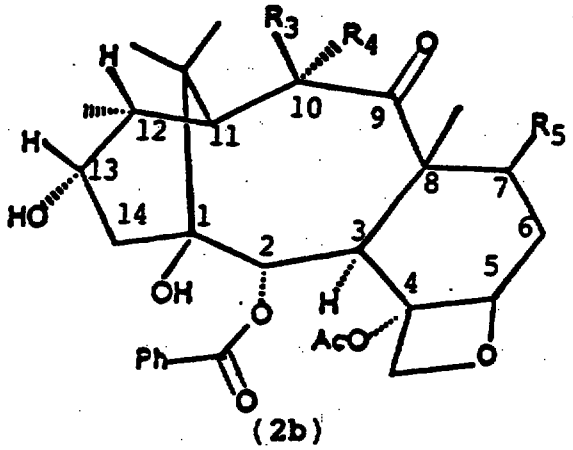
In particular, syntones of formula (2a) are used for the synthesis of the novel taxanes of formula (1a). On the other hand, syntones of formula (2b) are

employed for the synthesis of the novel taxanes of formula (1b).



(2a)

In syntones (2a), a double olefinic bond is present at the 11,12- position, and a C_2-C_8 acyloxy group or an optionally protected hydroxy group are present at the 10α - position. Therefore, in syntones (2a), R_3 is hydrogen, R_4 and R_5 are hydroxy, acyloxy, alkylsilyloxy (such as triethylsilyloxy, O-TES) or 2,2,2-trichloroethoxycarbonyloxy (O-CO-O-CH_2CCl_3, O-TROC) groups.



(2b)

In syntones (2b), the carbon atoms at the 11- and 12- positions are bonded by a single bond, the methyl at the 12- position is α -oriented, and an acyloxy group or an optionally protected hydroxy group are

present at the 10α - position. Therefore, in syntones (2b), R_4 is hydrogen, R_3 and R_5 are hydroxy, acyloxy, alkylsilyloxy (such as triethylsilyloxy, O-TES) or 2,2,2-trichloroethoxycarbonyloxy (O-CO-O-CH_2CCl_3, O-TROC) groups.

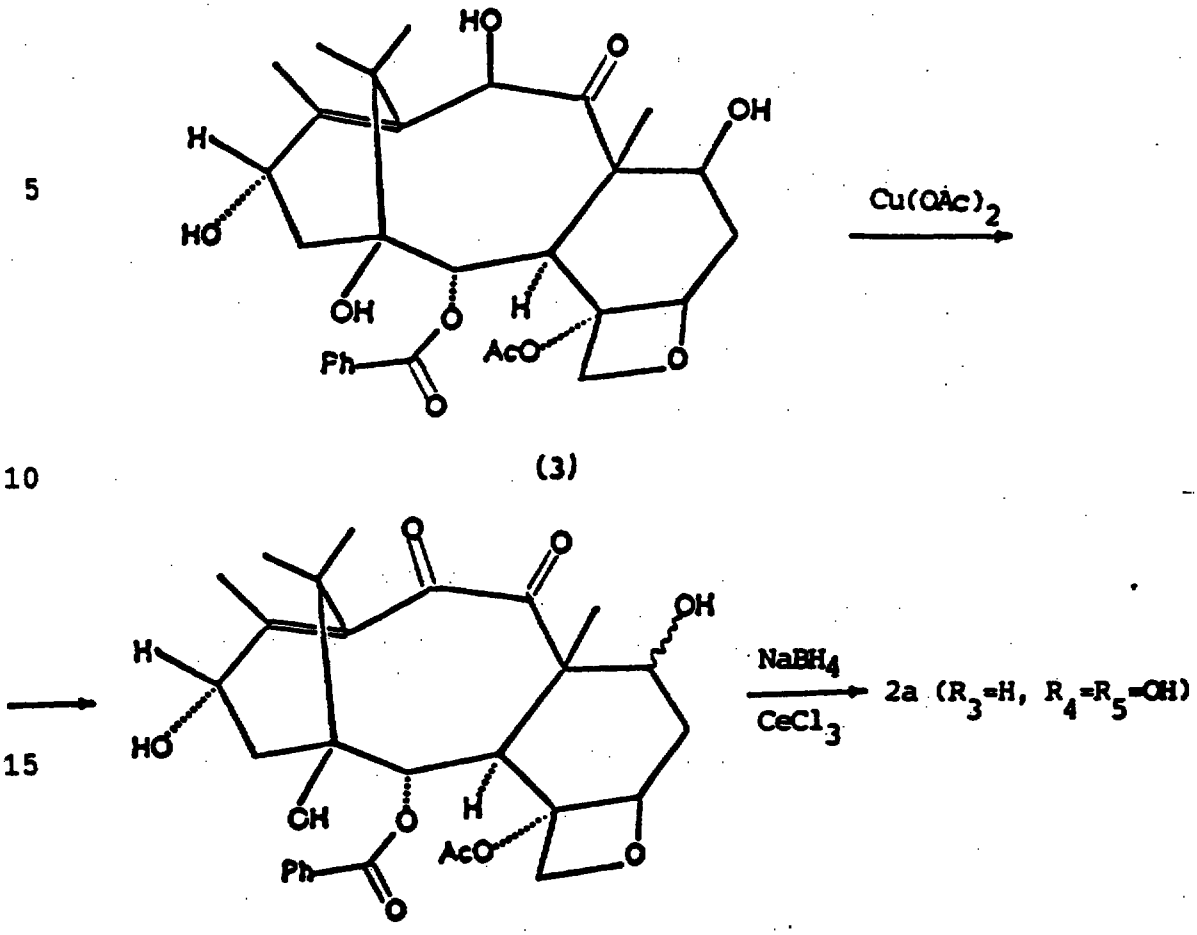
After esterificating at the 13- position the syntones (2) with the isoserine chain, the protective groups are removed by conventional methods known in literature, thereby obtaining the novel taxanes of Formula (1).

10-Deacetylbaccatine III (3), which can be isolated from the leaves of Taxus Baccata (G. Chauvière et coll., C.R. Acad. Sc. Ser. III, 293; 591 [1981]), is used as the sole starting product for the preparation of syntones (2a) and (2b).

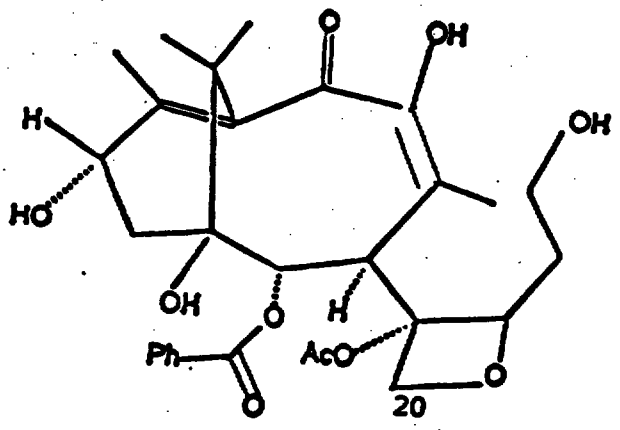
Syntones of formula (2a), which are not known in literature, are obtained (Scheme 1) from (3) by oxidation at the 10- position with copper (II) acetate, to give diketone (4), and subsequent reduction with sodium borohydride in the presence of cerium (III) salts.

The resulting product (2a, R_3 = H, R_4 = R_5 = OH), which is the epimer at the 10- position of (3), is suitably protected at the 7- and 10- positions and used for the synthesis of taxanes (1a).

Scheme 1



The new secotaxane (5) is obtained as a by-product of the reaction sequence given in Scheme 1

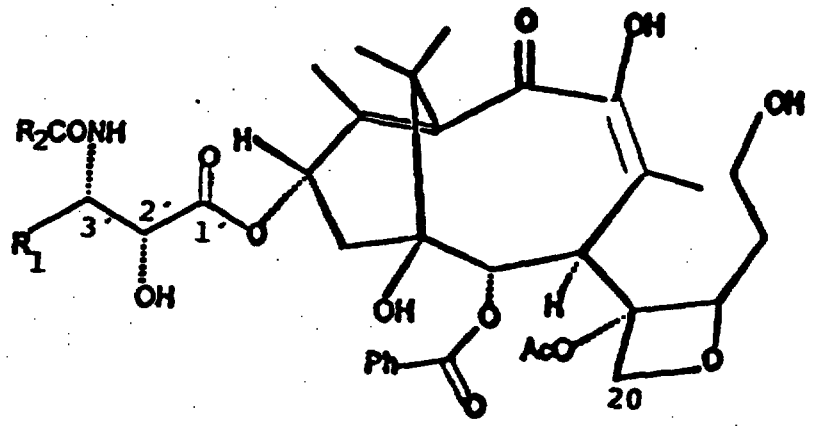


(5)

Secotaxane (5) can be used for the synthesis of

further taxanes with potential anti-tumoural activity.

The present invention also relates to novel secotaxane-skeleton derivatives prepared by semisynthesis and having a powerful anti-tumoural activity. Said derivatives have formula (5a)



(5a)

wherein:

R_1 and R_2, which can be the same or different, are a C_1-C_{20} alkyl, C_2-C_8 alkenyl, aryl (preferably phenyl) or heteroaryl groups. R_2 can also be tert-butoxy.

Taxanes of formula (5a) are prepared by esterificating compound of formula (5) in position 13, using the suitably activated isoserine chains as acylating agents, as reported in literature for the semisynthesis of taxol and analogues thereof (see for example EP-A- 400,971; E. Didier et al., Tetrahedron Letters 35, 2349, 1994; E. Didier at al., ibid. 35, 3063, 1994). The hydroxy groups of compound (5) can optionally be protected with suitable protective groups, according to conventional methods.

After the esterification of compound (5) at the 13- position with the isoserine chain, the protective

groups are removed according to conventional methods known in literature, thereby obtaining secotaxanes of formula (5a).

Syntones of formula (2b), which are not known in literature, are also obtained from 10-deacetylbaccatine III (3) (Scheme 2). It has been found that by oxidation of (3) with m-chloroperbenzoic acid (MCPBA), the corresponding 13-ketoderivative (6) is obtained. After protecting the hydroxyl at the 7- position with triethylsilyl chloride (TESCl), by reduction with sodium borohydride in the presence of cerium (III) salts, (6) gives syntone (2b) (R_3 = OH, R_4 = H, R_5 = O-TES), which can be useful for the synthesis of taxanes of formula (1b). The α -orientation of the methyl at the 12- position in syntones (2b) has been deduced by means of thorough studies using nuclear magnetic resonance.

Scheme 2

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The products of the present invention were screened for their cytotoxic effect on different tumour cell lines, comparing their action with that of taxol. Table 1 shows the IC_{50} data, compared with those found for taxol, of the compounds 13-[(2R, 3S)-3-phenyl-2-hydroxy-3-tert-butoxycarbonylamino-propanoyl]-10-epi-10-deacetylbaccatine III (1a, R_1 = Ph, R_2 =tBuO, R_3 = H, R_4 = OH), 13-[(2R, 3S)-3-phenyl-2-hydroxy-3-tert-butoxycarbonylamino-propanoyl]-10-deacetyl-11,12-dihydrobaccatine III (1b, R_1 = Ph, R_2 =tBuO, R_3 = OH, R_4 = H), 13-[(2R, 3S)-3-phenyl-2-hydroxy-3-tert-butoxy-carbonylamino-propanoyl]-C-seco-10-deacetylbaccatine III (5a, R_1 = Ph, R_2 = tBuO) and 13-[(2R, 3S)-3-isobutyl-2-hydroxy-3-caproylamino-propanoyl]-C-seco-10-deacetylbaccatine (5a, R_1 = isobutyl, R_2 = caproyl).

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Tab. 1 - IC_{50} of taxanes 1a (R_1 = Ph, R_2 = tBuO, R_3 = H, R_4 = OH), 1b (R_1 = Ph, R_2 = tBuO, R_3 = OH, R_4 = H) and of taxol on 6 cell lines.

	Exposure time (h)	Taxol	1a (R_1= Ph, R_2= tBuO, R_3= H, R_4= OH)	1b (R_1= Ph, R_2= tBuO, R_3= OH, R_4= H)
L1210 (murine leukemia)	48	57.0 ± 3.0	46.0 ± 2.1	32.0 ± 0.1
A121 (Human ovarian)	72	3.7 ± 0.3	2.8 ± 0.3	1.6 ± 0.2
A549 (Human NSCLC)	72	5.4 ± 0.5	6.9 ± 0.3	2.1 ± 0.3
HT-29 (Human colon)	72	6.0 ± 0.6	3.4 ± 0.1	3.6 ± 0.4
MCF7 (Human breast)	72	4.3 ± 0.1	2.2 ± 0.2	0.8 ± 0.2
MCF7-ADR (resistant)	72	395.0 ± 8.7	130.0 ± 2.2	128.0 ± 6.2

Standard conditions: substrate RPMI 1640 + 20 mM HEPES + 2 mM L-glutamine.

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Tab. 1 - IC_{50} of taxanes 5a (R_1 = Ph, R_2 = tBu0), 5a (R_1 = isobutyl, R_2 = caproyl) and of taxol on 6 cell lines.

	Exposure time (h)	Taxol	5a (R_1 = Ph, R_2 = tBu0)	5a (R_1 = isobutyl, R_2 = caproyl)
L1210 (murine leukemia)	48	57.0 ± 3.0	35 ± 1,2	26 ± 1,3
A121 (Human ovarian)	72	3.7 ± 0.3	1,9 ± 0,2	1,3 ± 0,1
A549 (Human NSCLC)	72	5.4 ± 0.5	3,3 ± 0,4	2,6 ± 0,3
HT-29 (Human colon)	72	6.0 ± 0.6	3,2 ± 0,3	2,7 ± 0,2
MCF7 (Human breast)	72	4.3 ± 0.1	1,5 ± 0,2	1,1 ± 0,2
MCF7-ADR (resistant)	72	395.0 ± 8.7	31,3 ± 4,2	25,4 ± 3,7

Standard conditions: substrate RPMI 1640 + 20 mM HEPES + 2 mM L-glutamine.

Compounds with different substituents at the isoserine chain behave similarly. The compounds show surprising advantages over taxol on the cell lines resistant to other anti-tumoural substances such as adriamycin and cis-platinum. The differences between taxol and these products are still more evident in in vivo models, such as athymic nude mouse with human tumour implant. It has also been found that the compounds of the invention in which R_2 is an alkyl or alkenyl group are surprisingly devoid of cardiotoxic activity, unlike taxol and its known derivatives, therefore they can favourably be used in cardiopathic patients untreatable with taxol and its known derivatives.

The compounds of the invention are suited for incorporation in appropriate pharmaceutical formulations for the parenteral and oral administrations. For the intravenous administration, mixtures of polyethoxylated castor oil and ethanol, or liposomal preparations prepared with natural phosphatidyl choline or mixtures of natural phospholipids in the presence of cholesterol, are mainly used.

The examples given below further illustrate the invention.

Example 1. Preparation of 10-dehydro-10-deacetylbaccatine III (4).

10 g of 10-deacetylbaccatine III (3) are suspended in 350 ml of methanol to which 65 g of $Cu(OAc)_2$ are added. The suspension is continuously stirred at room temperature for 120 hours. The salts are filtered off and the solution is chromatographed on 100 g of silica

gel eluting with a 6:4 hexane/ethyl acetate mixture. By crystallisation from ligroin, 9.5 g of (4) are obtained, M+ at m/z 542.

Example 2. Preparation of 10-deacetyl-10-epibaccatine III (2a, R_3=H, R_4=R_5=OH) and C-seco-10-deacetyl-baccatine III (5).

A solution of 300 mg of (4) in 5 ml of methanol is added with one equivalent of $CeCl_3.3H_2O$, stirred at room temperature for 5 minutes, then added with 80 mg of $NaBH_4$. The solution is treated with a NH_4Cl solution, extracted with ethyl acetate and chromatographed on silica gel eluting with a 3:7 hexane/ethyl acetate mixture. 98 mg of (2a) (M+ at m/z 544) and 120 mg of (5) (M+ at m/z 546) are obtained.

10-Deacetyl-10-epibaccatine III has the following ^1H-NMR spectrum ($CDCl^3$): H2, d 5.68 J 6.8; H3, d 4.26 J 6.8; H5, d 5.03 J 7.1; H7/13, m 4.76; H10, br s 5.20; 10 OH, br s 3.44; H16, s 1.14; H17, s 1.68; H18, s 2.22; H19, s 1.13; H20a, d 4.33; H20b, d 4.18; Ac, s 2.31; Bnz, br 8.12 J 8, br t 7.60 J 8, br t 17.49 J 8.

Example 3. Preparation of 10-deacetyl-13-dehydrobaccatine III (6)

3 g of meta-chloroperbenzoic acid and 1 g of sodium acetate are added to a suspension of 1 g of 10-deacetylbaccatine III (3) in 100 ml of CH_2Cl_2. The suspension is continuously stirred for 120 hours at room temperature and then diluted with a 5% Na_2CO_3 aqueous solution. The organic phase is washed with 5% Na_2CO_3 and evaporated to dryness. The residue is purified on silica gel eluting with a 3:7 hexane/ethyl acetate mixture. 789 mg of (6), M+ at m/z 542 are

obtained.

Example 4. Preparation of 10-deacetyl-11,12-dihydro-7-triethylsilyl-baccatine III (2b, R_3=OH, R_4=H, R_5=O-TES).

1.6 g of (6) are dissolved in methylene chloride and added with 370 mg of 4-dimethylaminopyridine and 2.5 ml of triethylsilyl chloride. After 2 hours at room temperature, the reaction mixture is diluted with methylene chloride and washed with water. The organic phase is concentrated to dryness. 1.72 g of a residue is obtained, which is taken up with 150 ml of 95% ethanol and treated with 9 g of $NaBH_4$. After 3 hours the mixture is diluted with a NH_4Cl solution and the product is extracted with ethyl acetate. Following chromatography on silica gel using a 7:3 hexane/ethyl acetate mixture, 800 mg of (2b) (R_3=OH, R_4=H, R_5=O-TES) are obtained.

Example 5. Preparation of 11,12-dihydro-7-TES-baccatine III (2b, R_3=OH, R_4=H, R_5=O-TES) and 11,12-dihydrobaccatine III (2b, R_3=OAc, R_4=H, R_5=OH)

500 mg of 10-deacetyl-11,12-dihydro-7-triethylsilylbaccatine III (2b, R_3=OH, R_4=H, R_5=O-TES) are reacted in anhydrous pyridine with 3 equivalents of acetyl chloride at 0°C for 6 hours. The reaction mixture is diluted with water and extracted with methylene chloride. After evaporation of the solvent, the residue is crystallised from acetone/hexane. 510 mg of 11,12-dihydro-7-TESbaccatine III are obtained, M+ at m/z 702 III. The product is dissolved in methanol and treated with diluted HCl until complete desilylation. The reaction mixture is diluted with water, extracted

with ethyl acetate and crystallisated from aqueous methanol. 400 mg of 11,12-dihydrobaccatine III are obtained, M+ at m/z 588.

Example 6. Preparation of 13-[(2R, 3S)-3-phenyl-2-hydroxy-3-tert-butoxycarbonylamino-propanoyl]-11,12-dihydrobaccatine III (1b, R_1=Ph, R_2=tBuO, R_3=OAc, R_4=H).

500 mg of 11,12-dihydrobaccatine III (2b, R_3=OAc, R_4=H, R_5=O-TES) are dissolved in 20 ml of toluene with 0.45 g of (4S, 5R)-N-tert-butoxycarbonyl-2,2-dimethyl-phenyl-5-oxazolydinecarboxylic acid, dicyclohexylcarbodiimide (1.03 eq) and N,N-dimethylaminopyridine (0.2 eq) at 80°C for 2 hours. The reaction mixture is washed with water until the excess of the reagents is removed, then concentrated to dryness. The residue is treated with methanol containing 1% formic acid for 4 hours at room temperature. The methanol solution is diluted with water, neutralised and extracted with ethyl acetate. The organic phase is concentrated to dryness and the residue is treated with a solution containing 1.5 eq of di-tert-butyl carbonate and sodium bicarbonate in 15 ml of tetrahydrofuran. The reaction mixture is diluted with water, extracted with ethyl acetate and the heteroacetic phase is concentrated to dryness. The residue is taken up with acidic methanol by hydrochloric acid to complete desilylation. The solution is then diluted with water and extracted with ethyl acetate. The residue obtained by evaporation of the heteroacetic phase is chromatographed on silica gel eluting with a 1:1 acetone/hexane mixture to remove the reaction impurities. 580 mg of product are obtained, M+ at m/z 851.

Example 7. Preparation of 13-[(2R, 3S)-3-benzoylamino-3-phenyl-2-hydroxypropanoyl]-11,12-dihydrobaccatine III (1b, R_1=R_2=Ph, R_3=OAc, R_4=H).

500 mg of 11,12-dihydro-7-TES-baccatine (2b, R_3=OAc, R_4=H, R_5=O-TES) are dissolved in 20 ml of toluene together with 1.5 g of (4S, 5R)-N-benzoyl-2,2-dimethyl-4-phenyl-5-oxazolydinecarboxylic acid, dicyclohexylcarbodiimide (1.03 eq) and N,N-dimethylaminopyridine (0.2 eq) at 80°C for 2 hours. The reaction mixture is washed with water until the excess of reagents is removed, then concentrated to dryness. The residue is treated with methanol containing 1% formic acid for 4 hours at room temperature. The methanol solution is diluted with water, neutralised and extracted with ethyl acetate. The organic phase is concentrated to dryness and the residue is taken up with methanol acidic by hydrochloric acid to complete desilylation. The solution is then diluted with water and extracted with ethyl acetate. The residue obtained by evaporation of the heteroacetic phase is chromatographed on silica gel eluting with a 1:1 acetone/hexane mixture to remove the reaction impurities. 530 mg of product are obtained, M+ at m/z 855.

Example 8. Preparation of 13-[(2R, 3S)-3-phenyl-2-hydroxy-3-tert-butoxycarbonylamino-propanoyl]-10-epi-10-deacetylbaccatine III (1a, R_1=Ph, R_2=tBuO, R_3=H, R_4=OH).

500 mg of 10-deacetyl-10-epibaccatine III (2a, R_3=H, R_4=R_5=OH) are dissolved in 15 ml of anhydrous pyridine and treated for 5 minutes at 80°C with three

equivalents of trichloroethoxycarbonyl chloride (TROC-Cl) and then cooled to room temperature. 1 ml of methanol is added to decompose the excess TROC-Cl. The solution is diluted with iced water and extracted with chloroform, washing the organic phase with a hydrochloric acid diluted solution. The organic phase is evaporated to dryness and the residue is treated at room temperature for 24 hours with a toluene solution containing three equivalents of (4S, 5R)-N-tert-butoxycarbonyl-2,2-dimethyl-4-phenyl-5-oxazolydinecarboxylic acid, 3 equivalents of dicyclohexylcarbodiimide and 0.2 equivalents of N,N-dimethylaminopyridine. The reaction mixture is washed with water and the organic phase is evaporated to dryness under vacuum. The residue is taken up with methanol and treated with one equivalent of p-toluenesulfonic acid for 48 hours, after that is diluted with water and extracted with ethyl acetate. The organic phase is evaporated under vacuum and the residue is taken up in 200 ml of a 1:1 acetic acid/ethyl acetate mixture and treated for 3 hours at 30°C with 11 equivalents of powdered zinc. The solid material is filtered off and the solution is diluted with water, extracted with ethyl acetate and chromatographed on silica gel eluting with a 1:4 ethyl acetate/hexane mixture. 512 mg of product (1a) are obtained, M+ at m/z 807.

Example 9. Preparation of 7,9-ditriethylsilyl-C-seco-10-deacetylbaccatine III

A solution of (5) (200 mg, 0.37 mmol) in anhydrous dimethylformamide (DMF) (5 ml), is added with imidazole (75 mg, 1.11 mmol, 3 eq. mol) and triethylsilyl

chloride (TES) (186 ml, 167.3 mg, 1.11 mmol, 3 eq. mol) and the reaction mixture is stirred for 10 minutes at room temperature. The reaction is checked by TLC (3:7 hexane-ethyl acetate, Rf of the starting material 0.10, Rf of the product 0.80). The reaction is quenched by addition of water and Celite^{R}, and the precipitate is filtered and washed with water to remove DMF, then with $CHCl_3$ to remove the product. After purification by column chromatography (9:1 hexane/ethyl acetate to elute silanol, then 6:4 hexane/ethyl acetate to elute the product) 146 mg of the title product are obtained (51%).

Example 10. Preparation of 13-[(2R,3S)-3-phenyl-2-hydroxy-3-tert-butoxycarbonylamino-propanoyl-C-seco-10-deacetylbaccatine III (5a, R_1=Ph, R_2=*t*BuO).

A solution of the product obtained in example 9 (126 mg, 0.16 mmol) in anhydrous toluene (5 ml), is added with 67.5 mg of dicyclohexylcarbodiimide (0.327 mmol, 2 mol. eq.), 105 mg of (4S,5R)-N-Boc-2-(2,4-dimethoxyphenyl)-4-phenyl-5-oxazolidinecarboxylic (0.327 mmol, 2 mol eq.) and 5 mg of 4-dimethylaminopyridine. The mixture is heated to 60°C for 24 hours and diluted with a $NaHCO_3$ saturated aqueous solution and ethyl acetate. The residue is purified by column chromatography (8:2 hexane-ethyl acetate) to give 175 mg of the 13-ester (95%). The residue is taken up with 50 ml of methanol/HCl (0.01%) and the reaction mixture is left at room temperature for 1 hour. The solution is alkalinized to pH 5 and concentrated to dryness under vacuum. The residue is chromatographed on a silica gel column eluting with a

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98:2 methylene chloride-methanol mixture. After crystallization from ethyl acetate, 85 mg of the title compound are obtained.

Example 11. Preparation of 13-[(2R,3S)-3-isobutyl-2-hydroxy-3-caproylamino-propanoyl-C-seco-10-deacetylbaccatine III (5a, R_1=Ph, R_2=caproyl).

A solution of the product obtained in example 9 (126 mg, 0.16 mmol) in anhydrous toluene (5 ml), is added with 67.5 mg of dicyclohexylcarbodiimide (0.327 mmol, 2 mol. eq.), 140 mg of (4S,5R)-N-caproyl-2-(2,4-dimethoxyphenyl)-4-isobutyl-5-oxazolidinecarboxylic acid (0,327 mmol, 2 mol eq.) and 5 mg of 4-dimethylaminopyridine. The mixture is heated to 60°C for 24 hours and diluted with a $NaHCO_3$ saturated aqueous solution and ethyl acetate. The residue is purified by column chromatography (8:2 hexane-ethyl acetate) to give 175 mg of the 13-ester (95%). The residue is taken up with 50 ml of methanol/HCl (0.01%) and the reaction mixture is left at room temperature for 1 hour. The solution is alkalinized to pH 5 and concentrated to dryness under vacuum. The residue is chromatographed on a silica gel column eluting with a 98:2 methylene chloride-methanol mixture. After crystallization from ethyl acetate, 88 mg of the title compound are obtained.

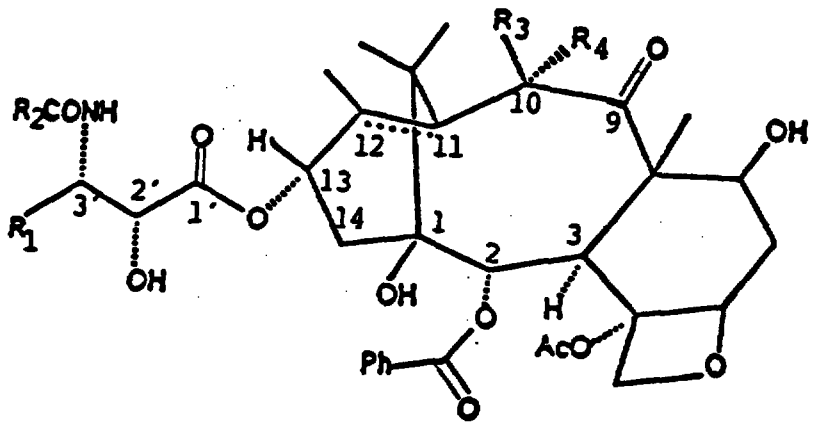
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CLAIMS

1. A semi-synthetic taxane of formula (1)



(1)

wherein:

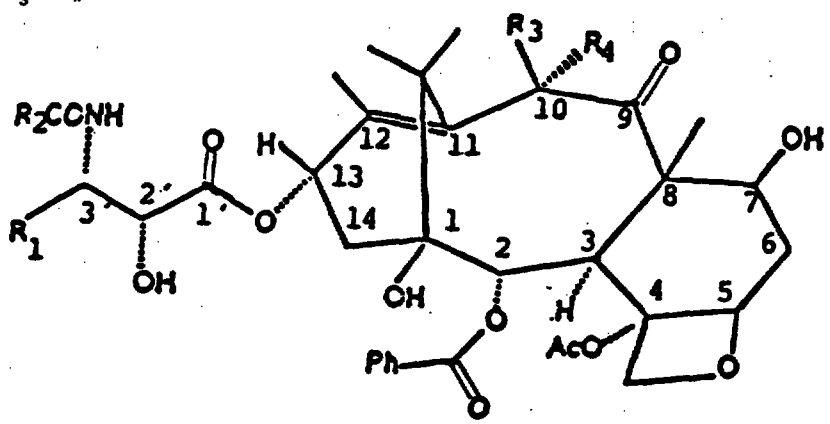
R_1 and R_2, which can be the same or different, are a C_1-C_8 alkyl, C_2-C_8 alkenyl, aryl or heteroaryl group; R_2 can be also a tert-butoxy group;

when a double olefinic bond is present in positions 11, 12, R_3 is a hydrogen atom, R_4 is a hydroxy or a C_2-C_8-acyloxy group;

when a double olefinic bond is not present in positions 11, 12, the methyl in position 12 has α -orientation, R_3 is a hydroxy or a C_2-C_8-acyloxy group, and R_4 is hydrogen.

2. As a compound according to claim 1, 13-[(2R, 3S)-3-phenyl-2-hydroxy-3-tert-butoxycarbonylamino-propanoyl]-10-epi-10-deacetylbaccatine III, of formula (1a)

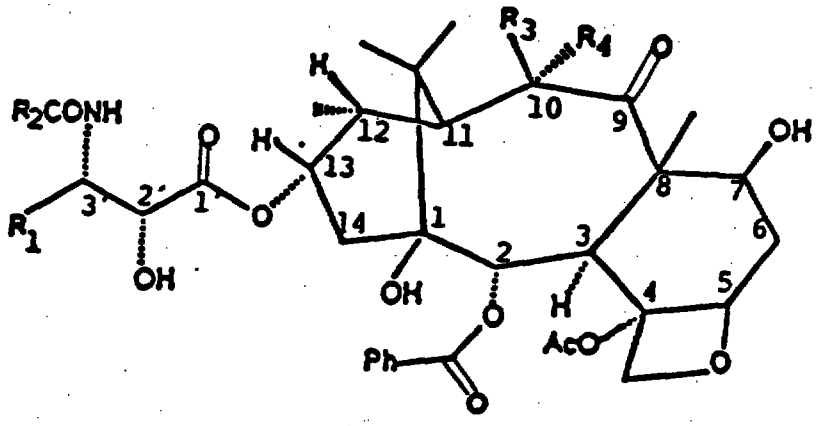
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(1a)

wherein R_1 = phenyl, R_2 = tert-butoxy, R_3 = H, R_4 = OH.

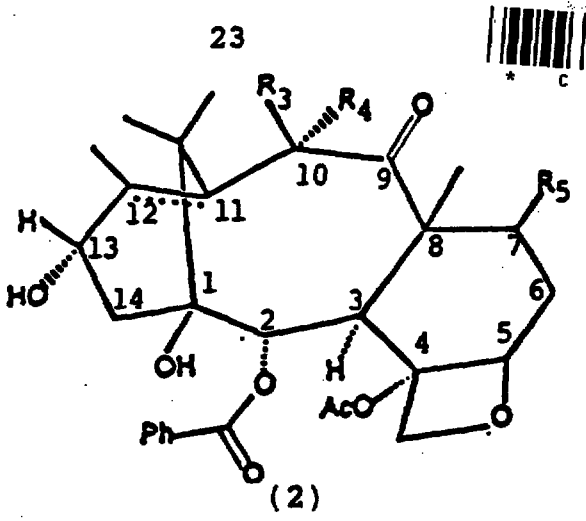
3. As a compound according to claim 1, 13-[(2R, 3S)-3-phenyl-2-hydroxy-3-tert-butoxycarbonylamino-propanoyl]-11,12-dihydrobaccatine III, of formula (1b)



(1b)

where R_1 = phenyl, R_2 = tert-butoxy, R_3 = acetoxy and R_4 = H.

4. A process for the preparation of the compounds according to claim 1, in which process syntones of formula (2)

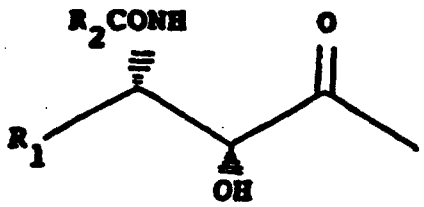


wherein:

when a double olefinic bond is present in position 11,12, R_3 is hydrogen, R_4 and R_5 are hydroxy, C_2-C_8-acyloxy, alkylsilyloxy or 2,2,2-trichloroethoxycarbonyloxy groups;

when a double olefinic bond is not present in position 11,12, the methyl in position 12 is α -oriented, R_4 is hydrogen, R_3 and R_5 are hydroxy, C_2-C_8-acyloxy, alkylsilyloxy or 2,2,2-trichloroethoxycarbonyloxy groups;

are subjected to esterification, according to known methods, with suitably activated and/or protected isoserine derivatives, thereby introducing the acyl group in position 13.

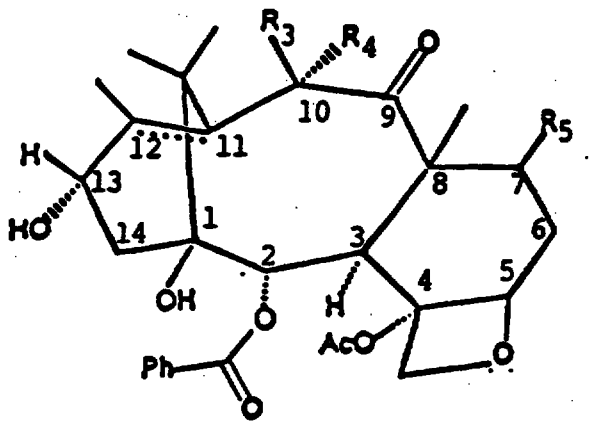


(where R_1 and R_2 have the meanings as in claim 1),

and the protective groups are subsequently removed according to known methods.

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5. As an intermediate, a compound of formula (2)



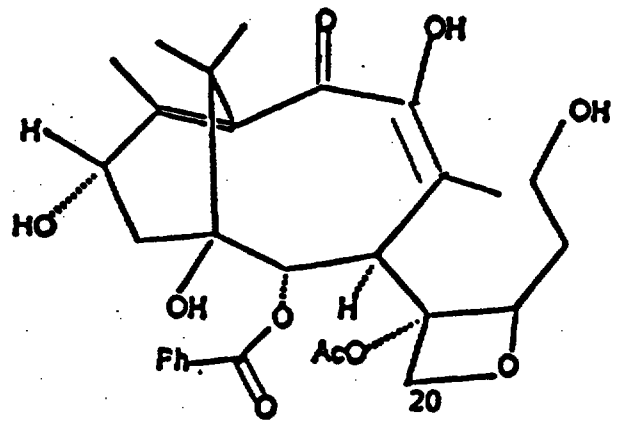
(2)

wherein:

when a double olefinic bond is present in position 11,12, R_3 is hydrogen, R_4 and R_5 are hydroxy, C_2-C_8-acyloxy, alkylsilyloxy or 2,2,2-trichloroethoxycarbonyloxy groups;

when a double olefinic bond is not present in position 11,12, the methyl in position 12 is α -oriented, R_4 is a hydrogen atom, R_3 and R_5 are hydroxy, C_2-C_8-acyloxy, alkylsilyloxy or 2,2,2-trichloroethoxycarbonyloxy groups.

6. As an intermediate, a compound of formula (5)

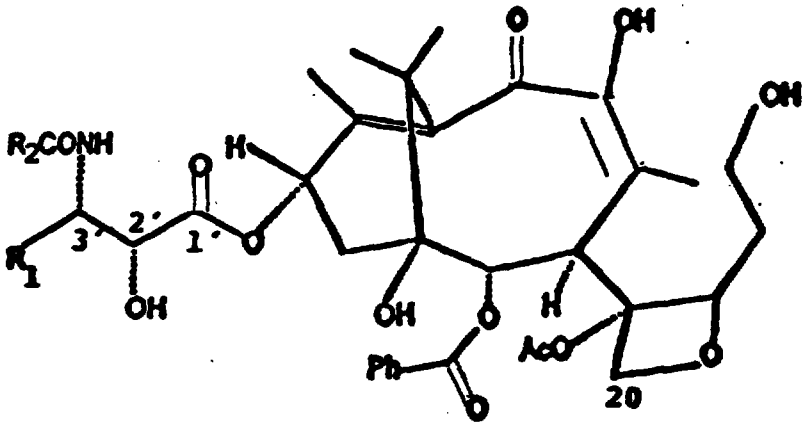


(5)

7. A semisynthetic secotaxane of formula 5a

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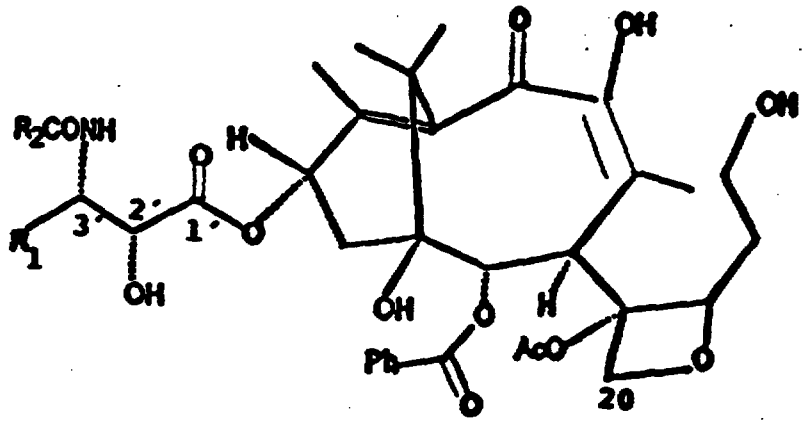


(5a)

wherein:

R_1 and R_2, which can be the same or different, are a C_1-C_{20} alkyl, C_2-C_8 alkenyl, aryl or heteroaryl group, R_2 can also be a tert-butoxy group.

8. As compound according to claim 7, 13-[(2R,3S)-3-phenyl-2-hydroxy-3-tert-butoxycarbonylamino-propanoyl]-C-seco-10-deacetylbaccatine III, of formula 5a



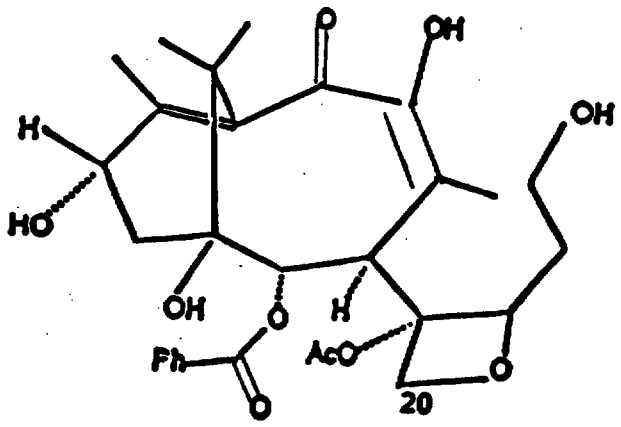
(5a)

wherein R_1 = phenyl, R_2 = tert-butoxy.

9. As compound according to the claim 7, 13-[(2R,3S)-3-isobutyl-2-hydroxy-3-caproylamino-propanoyl-C-seco-10-deacetylbaccatine III of formula 5a wherein R_1=isobutyl, R_2=caproyl.

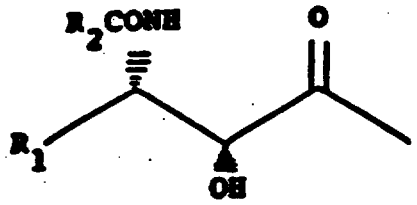
10. A process for the preparation of the compounds

according to claim 7, wherein compound of formula 5



(5)

is subjected to esterification, according to conventional methods, with derivatives suitably activated and/or protected at the isoserine chain, thereby introducing the acyl group in the 13-position



(wherein R_1 and R_2 have the meanings defined in claim 7),

thereafter removing the protective groups, according to conventional methods.

11. Pharmaceutical compositions containing taxanes according to claims 1-3 and 7-9.

12. Pharmaceutical compositions according to claim 11 having anti-tumoural action.

13. The use of taxanes according to claims 1-3 and 7-9 for the preparation of medicaments useful for the treatment of cancer.

14. The use of taxanes according to claim 1 or 7, wherein R_2 is an alkyl or alkenyl group, for the preparation of medicaments useful for the treatment of cancer in cardiopathic patients.

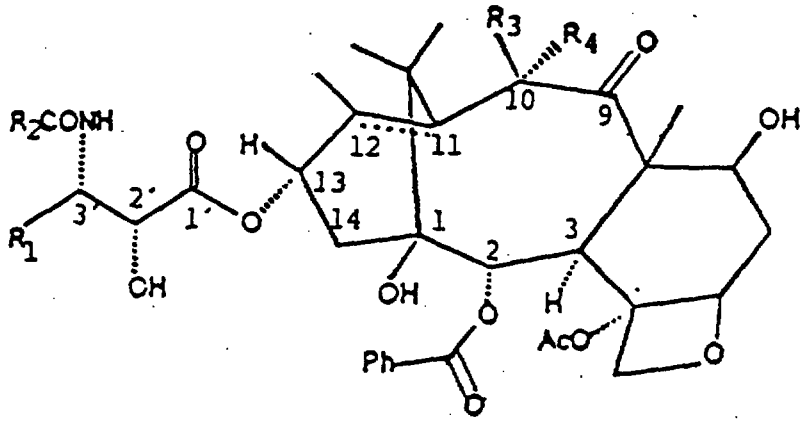
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SEMI-SYNTHETIC TAXANES WITH ANTI-TUMOURAL ACTIVITY

Diterpenes with taxane skeletons, and in particular taxol, are known to have an anti-tumoural action against numerous human tumours. However, the use of these drugs, particularly taxol, involves some drawbacks due to unwanted side effects. For this reason, and since these anti-tumoural treatments rapidly induce resistance, the development of new molecules whose use reduces the problems observed in clinical use is of interest.

* INSERT PAGE 1A

The present invention relates to novel taxane-skeleton derivatives obtained by semisynthesis and having a powerful anti-tumoural activity. The derivatives of the invention have formula (1):



(1)

They can be divided into two series:

a) taxane derivatives containing a double olefinic bond at the 11,12- position and a hydroxy or acyloxy group at the 10α-position (taxanes of formula 1a)

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WO 93/02067 (Nippon Steel) of February 4, 1993, discloses for istance 10-alfa-acetyl-taxol, extracted from tissue culture of albumen of Taxus species, having a marked cytotoxic activity.

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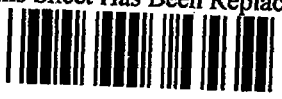
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The products of the present invention were screened for their cytotoxic effect on different tumour cell lines, comparing their action with that of taxol. Table 1 shows the IC_{50} data, compared with those found for taxol, of the compounds 13-[(2R, 3S)-3-phenyl-2-hydroxy-3-tert-butoxycarbonylamino-propanoyl]-10-epi-10-deacetylbaccatine III (1a, R_1 = Ph, R_2 =tBuO, R_3 = H, R_4 = OH), 13-[(2R, 3S)-3-phenyl-2-hydroxy-3-tert-butoxycarbonylamino-propanoyl]-10-deacetyl-11,12-dihydrobaccatine III (1b, R_1 = Ph, R_2 =tBuO, R_3 = OH, R_4 = H), 13-[(2R, 3S)-3-phenyl-2-hydroxy-3-tert-butoxy-carbonylamino-propanoyl]-C-seco-10-deacetylbaccatine III (5a, R_1 = Ph, R_2 = tBuO) and 13-[(2R, 3S)-3-isobutyl-2-hydroxy-3-caproylamino-propanoyl]-C-seco-10-deacetylbaccatine (5a, R_1 = isobutyl, R_2 = PENTYL caproyl).

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Tab. 1 - IC_{50} of taxanes 5a (R_1 = Ph, R_2 = tBuO), 5a (R_1 = isobutyl, R_2 = ~~caproyl~~ PENTYL) and of taxol on 6 cell lines.

	Exposure time (h)	Taxol	5a (R_1 = Ph, R_2 = tBuO)	5a (R_1 = isobutyl, R_2 = ~~caproyl~~ PENTYL)
L1210 (murine leukemia)	48	57.0 ± 3.0	35 ± 1,2	26 ± 1,3
A121 (Human ovarian)	72	3.7 ± 0.3	1,9 ± 0,2	1,3 ± 0,1
A549 (Human NSCLC)	72	5.4 ± 0.5	3,3 ± 0,4	2,6 ± 0,3
HT-29 (Human colon)	72	6.0 ± 0.6	3,2 ± 0,3	2,7 ± 0,2
MCF7 (Human breast)	72	4.3 ± 0.1	1,5 ± 0,2	1,1 ± 0,2
MCF7-ADR (resistant)	72	395.0 ± 8.7	31,3 ± 4,2	25,4 ± 3,7

Standard conditions: substrate RPMI 1640 + 20 mM HEPES + 2 mM L-glutamine.

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98:2 methylene chloride-methanol mixture. After crystallization from ethyl acetate, 85 mg of the title compound are obtained.

Example 11. Preparation of 13-[(2R,3S)-3-isobutyl-2-hydroxy-3-caproylamino-propanoyl-C-seco-10-deacetylbaccatine III (5a, R_1=Ph, R_2=~~caproyl~~ PENTYL).

A solution of the product obtained in example 9 (126 mg, 0.16 mmol) in anhydrous toluene (5 ml), is added with 67.5 mg of dicyclohexylcarbodiimide (0.327 mmol, 2 mol. eq.), 140 mg of (4S,5R)-N-caproyl-2-(2,4-dimethoxyphenyl)-4-isobutyl-5-oxazolidinecarboxylic acid (0,327 mmol, 2 mol eq.) and 5 mg of 4-dimethylaminopyridine. The mixture is heated to 60°C for 24 hours and diluted with a $NaHCO_3$ saturated aqueous solution and ethyl acetate. The residue is purified by column chromatography (8:2 hexane-ethyl acetate) to give 175 mg of the 13-ester (95%). The residue is taken up with 50 ml of methanol/HCl (0.01%) and the reaction mixture is left at room temperature for 1 hour. The solution is alkalinized to pH 5 and concentrated to dryness under vacuum. The residue is chromatographed on a silica gel column eluting with a 98:2 methylene chloride-methanol mixture. After crystallization from ethyl acetate, 88 mg of the title compound are obtained.

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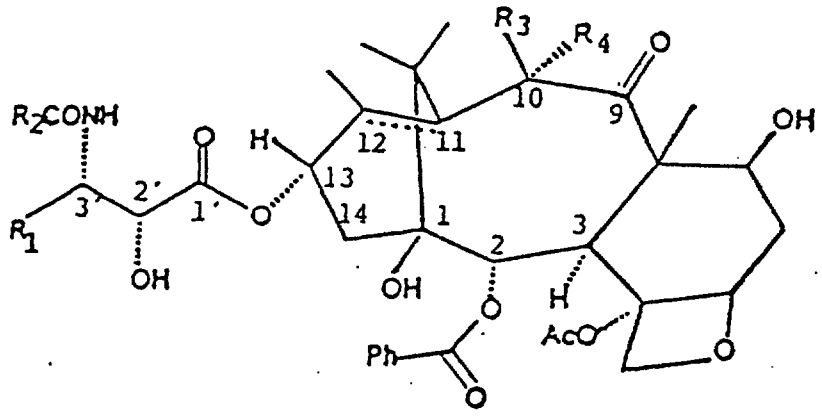
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CLAIMS

1. A semi-synthetic taxane of formula (1)



(1)

wherein:

R_1 and R_2, which can be the same or different, are a C_1-C_8 alkyl, C_2-C_8 alkenyl, aryl or heteroaryl group;

R_2 can be also a tert-butoxy group;

when a double olefinic bond is present in positions 11, 12, R_3 is a hydrogen atom, R_4 is a hydroxy or a C_2-C_8-acyloxy group;

when a double olefinic bond is not present in positions 11, 12, the methyl in position 12 has α -orientation, R_3 is a hydroxy or a C_2-C_8-acyloxy group, and R_4 is hydrogen. * INSERT PAGE 21A

2. As a compound according to claim 1, 13-[(2R, 3S)-3-phenyl-2-hydroxy-3-tert-butoxycarbonylamino-propanoyl]-10-epi-10-deacetylbaccatine III, of formula (1a)

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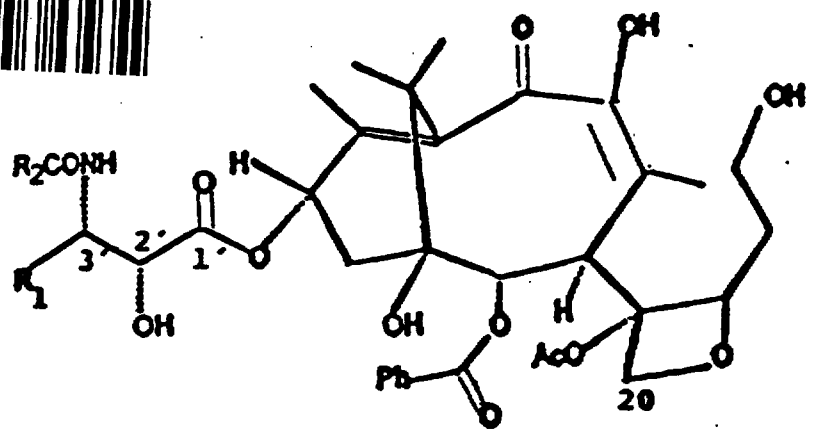
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With the proviso that R_1 and R_2 are phenyl and a double bond connects positions 11 and 12, R_4 is not acetyl.

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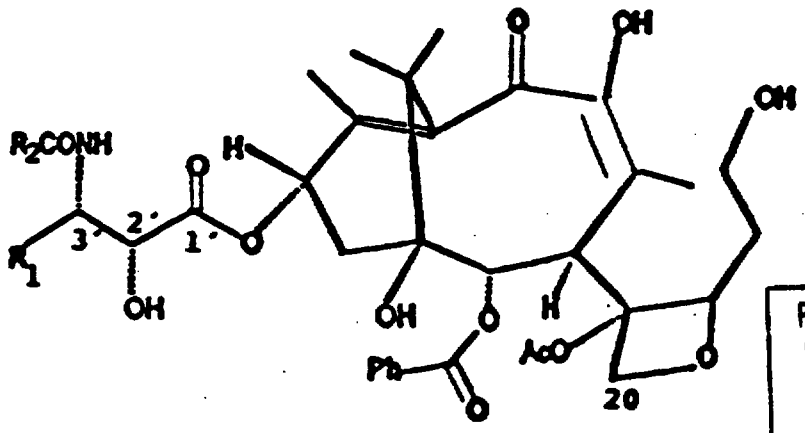


(5a)

wherein:

R_1 and R_2, which can be the same or different, are a C_1-C_{20} alkyl, C_2-C_8 alkenyl, aryl or heteroaryl group; R_2 can also be a tert-butoxy group.

8. As compound according to claim 7, 13-[(2R,3S)-3-phenyl-2-hydroxy-3-tert-butoxycarbonylamino-propanoyl]-C-seco-10-deacetylbaccatine III, of formula 5a



(5a)

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wherein R_1 = phenyl, R_2 = tert-butoxy.

9. As compound according to the claim 7, 13-[(2R,3S)-3-isobutyl-2-hydroxy-3-caproylamino-propanoyl-C-seco-10-deacetylbaccatine III of formula 5a wherein R_1=isobutyl, R_2=caproyl.

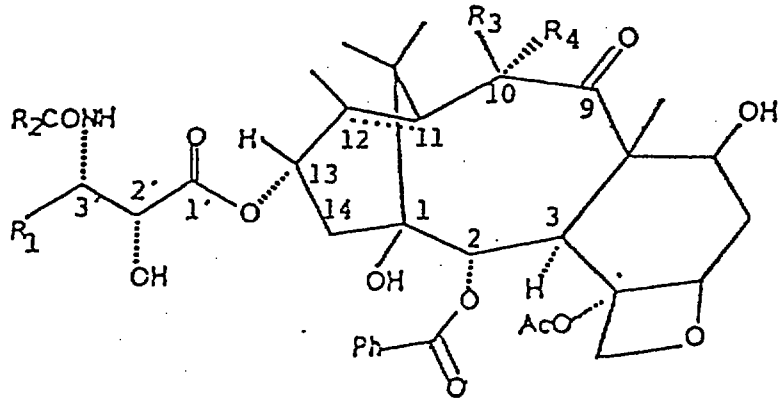
10. A process for the preparation of the compounds

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SEMI-SYNTHETIC TAXANES WITH ANTI-TUMOURAL ACTIVITY

Diterpenes with taxane skeletons, and in particular taxol, are known to have an anti-tumoural action against numerous human tumours. However, the use of these drugs, particularly taxol, involves some drawbacks due to unwanted side effects. For this reason, and since these anti-tumoural treatments rapidly induce resistance, the development of new molecules whose use reduces the problems observed in clinical use is of interest. WO 93/02067 (Nippon Steel) of February 4, 1993, discloses for instance 10-alfa-acetyl-taxol, extracted from tissue culture of albumen of Taxus species, having a marked cytotoxic activity.

The present invention relates to novel taxane-skeleton derivatives obtained by semisynthesis and having a powerful anti-tumoural activity. The derivatives of the invention have formula (1):

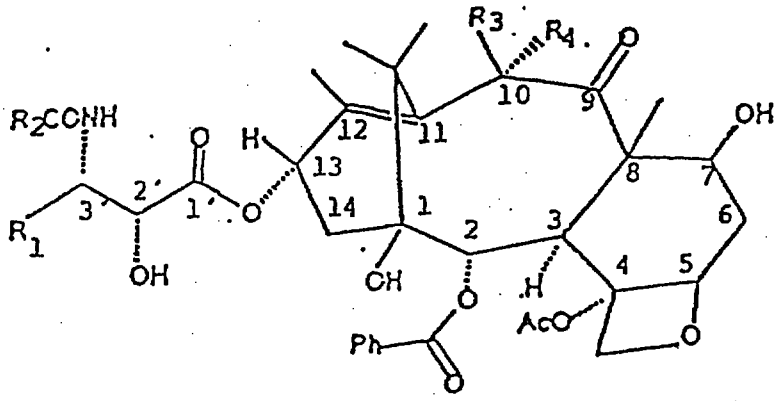


(1)

They can be divided into two series:

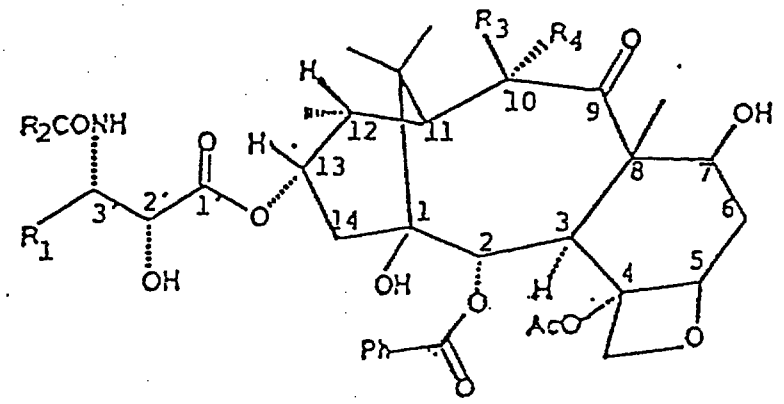
a) taxane derivatives containing a double olefinic bond at the 11,12- position and a hydroxy or acyloxy group at the 10α-position (taxanes of

1997



(1a) (R_3 = H; R_4 = OH or acyloxy)

b) taxane derivatives containing a single bond between the carbon atoms in 11 and 12, the methyl in 12 being α-oriented, and a hydroxy or acyloxy group in position 10β (taxane of formula 1b)



(1b) (R_3 = OH or acyloxy; R_4 = H)

In taxanes of general formula (1), R_1 and R_2, which may be the same or different, are a C_1-C_8 alkyl, C_2-C_8 alkenyl, aryl (preferably phenyl) or heteroaryl group. R_2 can also be a tert-butoxy group.

In compounds of formula (1a), R_3 is hydrogen and R_4 is a hydroxy or C_2-C_8 acyloxy group, with the proviso that when R_1 and R_2 are phenyl, R_4 is not acetyl.

In compounds of formula (1b), R_3 is a hydroxy or C_2-C_8 acyloxy group and R_4 is hydrogen.

Taxanes of formula (1) are prepared by

Date : 02 SEP 1997

The products of the present invention were screened for their cytotoxic effect on different tumour cell lines, comparing their action with that of taxol. Table 1 shows the IC_{50} data, compared with those found for taxol, of the compounds 13-[(2R, 3S)-3-phenyl-2-hydroxy-3-tert-butoxycarbonylamino-propanoyl]-10-epi-10-deacetylbaccatine III (1a, R_1 = Ph, R_2 =tBuO, R_3 = H, R_4 = OH), 13-[(2R, 3S)-3-phenyl-2-hydroxy-3-tert-butoxycarbonylamino-propanoyl]-10-deacetyl-11,12-dihydrobaccatine III (1b, R_1 = Ph, R_2 =tBuO, R_3 = OH, R_4 = H), 13-[(2R, 3S)-3-phenyl-2-hydroxy-3-tert-butoxy-carbonylamino-propanoyl]-C-seco-10-deacetylbaccatine III (5a, R_1 = Ph, R_2 = tBuO) and 13-[(2R, 3S)-3-isobutyl-2-hydroxy-3-caproylamino-propanoyl]-C-seco-10-deacetylbaccatine (5a, R_1 = isobutyl, R_2 = pentyl).

Tab. 1 - IC_{50} of taxanes 5a (R_1 = Ph, R_2 = tBuO), 5a (R_1 = isobutyl, R_2 = pentyl.) and of taxol on 6 cell lines.

	Exposure time (h)	Taxol	5a (R_1 = Ph, R_2 = tBuO)	5a (R_1 = isobutyl, R_2 = pentyl.)
L1210 (murine leukemia)	48	57.0 ± 3.0	35 ± 1,2	26 ± 1,3
A121 (Human ovarian)	72	3.7 ± 0.3	1,9 ± 0,2	1,3 ± 0,1
A549 (Human NSCLC)	72	5.4 ± 0.5	3,3 ± 0,4	2,6 ± 0,3
HT-29 (Human colon)	72	6.0 ± 0.6	3,2 ± 0,3	2,7 ± 0,2
MCF7 (Human breast)	72	4.3 ± 0.1	1,5 ± 0,2	1,1 ± 0,2
MCF7-ADR (resistant)	72	395.0 ± 8.7	31,3 ± 4,2	25,4 ± 3,7

Standard conditions: substrate RPMI 1640 + 20 mM HEPES + 2 mM L-glutamine.

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98:2 methylene chloride-methanol mixture. After crystallization from ethyl acetate, 85 mg of the title compound are obtained.

Example 11. Preparation of 13-[(2R,3S)-3-isobutyl-2-hydroxy-3-caproylamino-propanoyl-C-seco-10-deacetylbaccatine III (5a, R_1=Ph, R_2=pentyl).

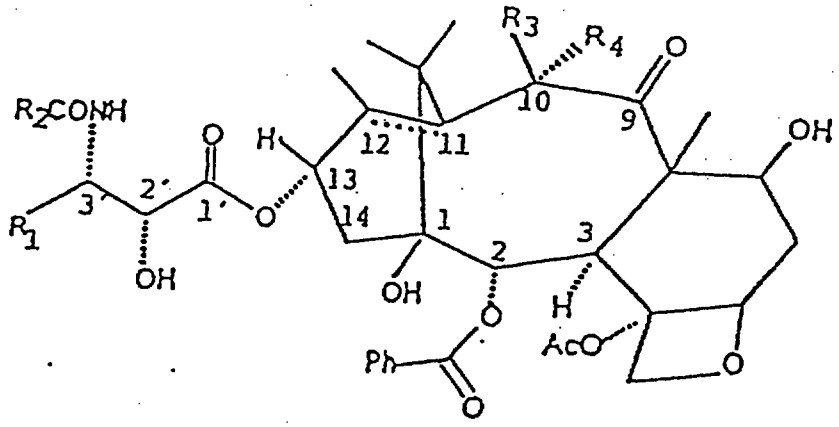
A solution of the product obtained in example 9 (126 mg, 0.16 mmol) in anhydrous toluene (5 ml), is added with 67.5 mg of dicyclohexylcarbodiimide (0.327 mmol, 2 mol. eq.), 140 mg of (4S,5R)-N-caproyl-2-(2,4-dimethoxyphenyl)-4-isobutyl-5-oxazolidinecarboxylic acid (0,327 mmol, 2 mol eq.) and 5 mg of 4-dimethylaminopyridine. The mixture is heated to 60°C for 24 hours and diluted with a $NaHCO_3$ saturated aqueous solution and ethyl acetate. The residue is purified by column chromatography (8:2 hexane-ethyl acetate) to give 175 mg of the 13-ester (95%). The residue is taken up with 50 ml of methanol/HCl (0.01%) and the reaction mixture is left at room temperature for 1 hour. The solution is alkalinized to pH 5 and concentrated to dryness under vacuum. The residue is chromatographed on a silica gel column eluting with a 98:2 methylene chloride-methanol mixture. After crystallization from ethyl acetate, 88 mg of the title compound are obtained.

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CLAIMS

1. A semi-synthetic taxane of formula (1)



(1)

wherein:

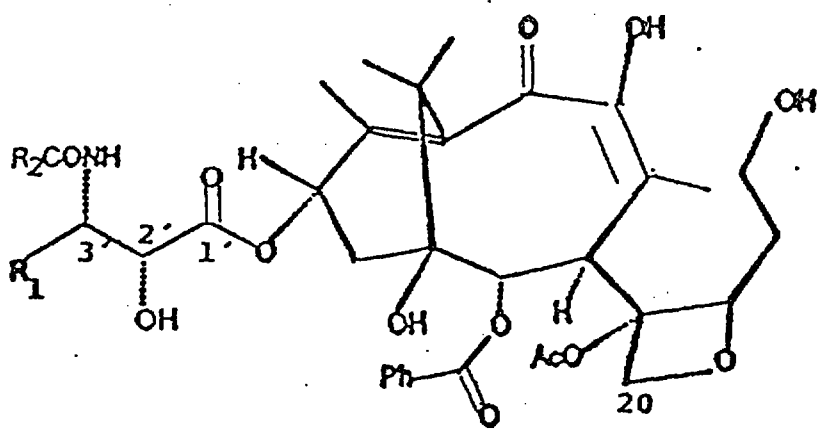
R_1 and R_2, which can be the same or different, are a C_1-C_8 alkyl, C_2-C_8 alkenyl, aryl or heteroaryl group; R_2 can be also a tert-butoxy group;

when a double olefinic bond is present in positions 11, 12, R_3 is a hydrogen atom, R_4 is a hydroxy or a C_2-C_8-acyloxy group;

when a double olefinic bond is not present in positions 11, 12, the methyl in position 12 has α -orientation, R_3 is a hydroxy or a C_2-C_8-acyloxy group, and R_4 is hydrogen, with the proviso that when R_1 and R_2 are phenyl and a double bond connects positions 11 and 12, R_4 is not acetyl.

2. As a compound according to claim 1, 13-[(2R, 3S)-3-phenyl-2-hydroxy-3-tert-butoxycarbonylamino-propanoyl]-10-epi-10-deacetylbaccatine III, of formula (1a)

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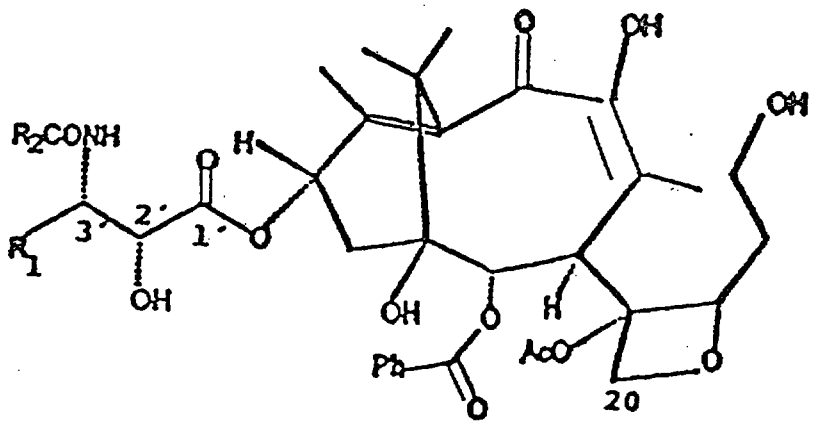


(5a)

wherein:

R_1 and R_2, which can be the same or different, are a C_1-C_{20} alkyl, C_2-C_8 alkenyl, aryl or heteroaryl group, R_2 can also be a tert-butoxy group.

8. As compound according to claim 7, 13-[(2R,3S)-3-phenyl-2-hydroxy-3-tert-butoxycarbonylamino-propanoyl]-C-seco-10-deacetylbaccatine III, of formula 5a



(5a)

wherein R_1 = phenyl, R_2 = tert-butoxy.

9. As compound according to the claim 7, 13-[(2R,3S)-3-isobutyl-2-hydroxy-3-caproylamino-propanoyl-C-seco-10-deacetylbaccatine III of formula 5a wherein R_1=isobutyl, R_2= pentyl.

10. A process for the preparation of the compounds