(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
29 August 2002 (29.08.2002)

PCT

(10) International Publication Number
WO 02/066448 A1

(51) International Patent Classification[7]: C07D 263/06

(21) International Application Number: PCT/IN01/00020

(22) International Filing Date: 20 February 2001 (20.02.2001)

(25) Filing Language: English

(26) Publication Language: English

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/066448 A1

(54) Title: METHOD OF PREPARATION OF PACLITAXEL (TAXOL) USING 3-(ALK-2-YNYLOXY) CARBONYL-5-OXAZOLIDINE CARBOXYLIC ACID

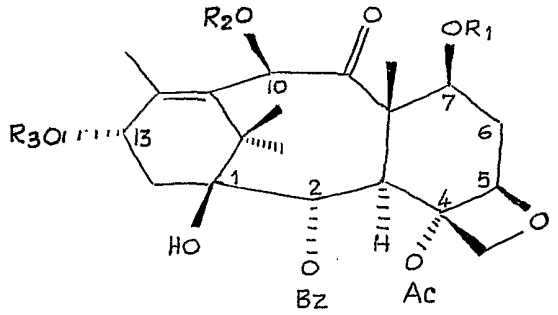
(57) Abstract: 3-(alk-2-ynyloxy) carbonyl- 5-oxazolidine carboxylic acid and its analogs having formula (8) and wherein R_1 is hydrogen, aryl, heteroaryl, alkyl, alkenyl, alkynyl, R_2 and R_3 are independently selected from hydrogen, alkyl, alkenyl, alkynyl, aryl, heteroaryl, R_4 is hydrogen, alkyl, alkenyl, alkynyl, aryl, substituted aryl, heteroaryl, R_5 and R_6 independently selected from hydrogen, alkyl, alkenyl, alkynyl, aryl, heteroaryl, alkoxy, alkeyloxy, alkynyloxy, aryloxy, heteroaryloxy.

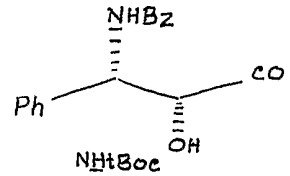
TITLE

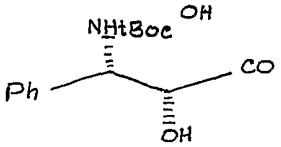
Method of preparation of Paclitaxel (Taxol) using 3-(alk-2-ynyloxy) carbonyl-5-oxazolidine carboxylic acid.

FIELD OF THE INVENTION

The present invention relates to a novel oxazolidine carboxylic acid and a process for its preparation. The invention further relates to a process for the preparaton of paclitaxel (taxol) using such oxazolidine carboxylic acid. Paclitaxel (Taxol) (1) is a terpene of taxane family and has the formula shown below and has an application for treatment of various types of cancer. Non-natural analog of paclitaxel (taxol), docetaxel (taxotere) (2) is also an approved anticancer drug. Thus, there is great interest in molecules having similar structures for development of new anticancer drugs and SAR studies.



1. Taxol R_1=H, R_2=Ac, R_3= 

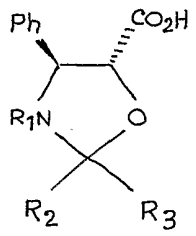
2. Taxotere R_1=R_2=H, R_3= 

3. Baccatin R_1=R_3=H, R_2=Ac
4. 10-Deacetylbacctin (DAB) R_1=R_2=R_3=H

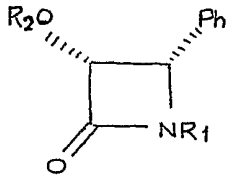
BACKGROUND OF THE INVENTION

The structure of paclitaxel (taxol) has two distinct unts. One is baccatin (3) and other is N-benzoylphenylisoserine, the side chain, connected to baccatin at C-13 through ester linkage. It has been shown that the β-amido-α-hydroxy ester side chain at C-13 is very essential for anticancer activity of taxol and any other taxol analogs. The supply of taxol from natural sources is very limited and so there is great interest in its synthesis. 10-deacetyl baccatin (4) is more readily available than taxol from the leaves of yew tree and comprises a starting material for semisynthesis of taxol and taxol analogs. It is known in the literature that it is very difficult to esterify 13 hydroxy of bacctin with β-amido-α-hydroxy carboxylic acid. The difficulty has been ascribed to spatial disposition of 13 hydroxy in the baccatin nucleus. Therefore, considerable efforts have been made to find precursors of β-amido-α-hydroxy carboxylic acid which can be coupled with bacctin in high yield and the resultant couple product can be processed to afford 13-O- (β-amido-α-hydroxycarbonyl) baccatin in high yield and purity.

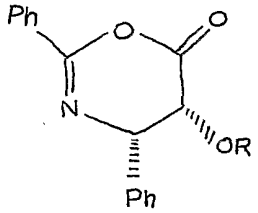
The following types of cyclic derivative of phenylisoserine are known to couple with 13-hydroxy of baccatin.



5



6



7

R, R1, R2, and R3 = Protecting groups

In the literature, various protecting groups, R_1, are described in oxazolidinecarboxylic acid (5). However, alk-2-ynyloxycarbonyl group have not been described so far. The utility of this group over other commonly used protecting groups lies in the fact that this group can be cleaved under neutral condition. All other known protecting groups (R_1) are cleaved under either acidic conditions or by hydrogenolysis. The subsequent cleavage of protecting groups R_2 and R_3 are normally very fast, once R_1 is cleaved. Since the baccatin part of taxol is very prone to degradation even under mild acidic or basic conditions, removal of alk-2-ynyloxycarbonyl group under neutral condition facilitates the conversion of taxol intermediate such as 10 to taxol in high yield and high purity.

OBJECTS OF THE INVENTION

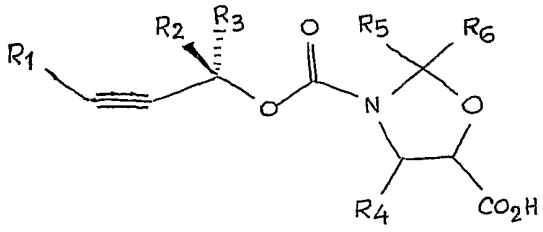
An object of this invention is to propose a novel oxazolidine carboxylic acid and a process for the preparation thereof.

Another object of this invention is to propose oxazolidine carboxylic acid which can advantageously be used in the preparation of anticancer drugs.

Still another object of this invention is to propose a process for the preparation of taxol and its synthetic analogs using the proposed oxazolidine carboxylic acid.

DESCRIPTION OF THE INVENTION

The present invention is directed to 3-(alk-2-ynyloxy) carbonyl- 5-oxazolidine carboxylic acid and its analogs having a formula



8

and wherein R_1 is hydrogen, aryl, heteroaryl, alkyl, alkenyl, alkynyl, R_2 and R_3 are independently selected from hydrogen, alkyl, alkenyl, alkynyl, aryl, heteroaryl; R_4 is hydrogen, alkyl, alkenyl, alkynyl, aryl, substituted aryl, heteroaryl.

R_5 and R_6 independently selected from hydrogen, alkyl, alkenyl, alkynyl, arly, heteroaryl, alkoxy, alkeyloxy, alkynyloxy, aryloxy, heteroaryloxy.

The oxazoldine alkyl groups either alone or with the variable substituents defined above are preferably lower alkyl containing from one to six carbon atoms in the principal chain and up to 10 carbon atoms. They may be straight or branched chain and include methyl, ethyl, propyl, isopropyl, butyl, isobutyl, tert-butyl, pentyl, hexyl and the like. The alkyl part of alkoxy groups defined above are same as a alkyl groups.

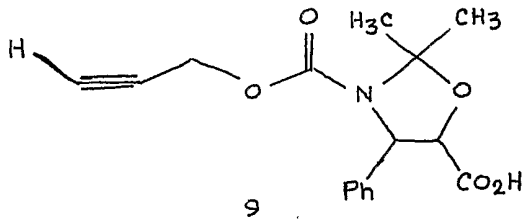
The oxazoldine alkenyl groups either alone or with the various substituents defined above are preferably lower alkenyl containing from two to six carbon atoms in the principal chain and up to 10 carbon atoms. They may be straight chain and include ethenyl, propenyl, isopropenyl, butenyl, isobutenyl, pentenyl, hexenyl, and the like. The alkenyl part of alkenyloxy groups defined above are same as a alkenyl groups.

The oxazoldine alknyl groups, either along or with the various substituents defined above are preferably lower alkynyl containing from two to six carbon atoms in the principal chain and up to 10 carbon atoms. They may be straight chain and include ethynyl, propynyl, butynyl, isobutynyl, pentynyl, hexynyl and the like. The alkynyl part of alkynyloxy groups defined above are same as alkynyl groups.

The oxazoldine aryl moieties eiher alone or with various substituents contain from 6 to 10 carbon atoms and include pheny, α- naphthyl etc. substituents include alkanoxy, hydroxy, halogen, alkyl, aryl, alkenyl, acyl, acyloxy, nitro, amino; amido etc.

As defined herein, the term aryloxy includes aromatic heterocyclic moieties the term aryl includes any compound having an aromatic ring of which no hetero atom is a member, and the term heteroaryl includes any compound having an aromatic ring which comprises a hetero atom.

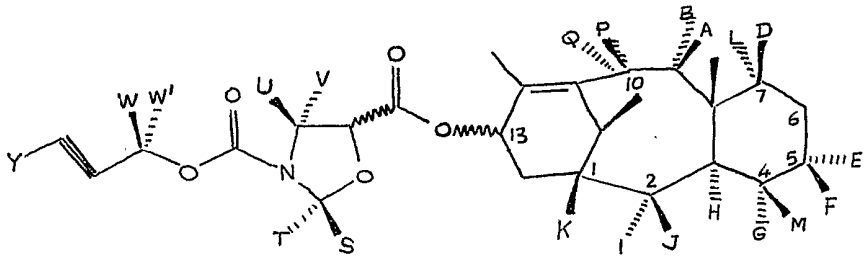
Most preferably, the oxazolidine carboxylic acid has a formula wherein R_1, R_2 and R_3 are hydrogen, R_4 is phenyl and R_5 and R_6 are methyl.



9

The present invention also describes process of preparation of 3-(alk-2-ynyloxy)carbonyl- 5-oxazolidine carboxylic acid and its analogs.

The present invention also describes the preparation of taxol intermediates of following structural formula:



10

wherein

A and B are independently hydrogen or lower alkanoyloxy, alkenoyloxy, alkynoyloxy aryloyloxy or;

A and B together form an oxo;

L and D are independently hydrogen or hydroxy or lower alkanoyloxy, alkenoyloxy, alkynoyloxy, aryloyloxy, alkoxycarbonyloxy, aryloxycarbonyloxy or alkylsilyloxy;

E and F are independently hydrogen or lower alkanoyloxy, alkenoyloxy, alkynoyloxy aryloyloxy; or E and F together form an oxo;

G is hydrogen or hydroxy or lower alkanoyloxy, alkenoyloxy, alkynoyloxy, or aryloyloxy or;

G and M together form an oxo or methylene or oxirane ring or

M and F together form an oxetane ring;

J is hydrogen or hydroxy or lower alkanoyloxy, alkenoyloxy, alkynoyloxy, or aryloyloxy or

I is hydrogen or hydroxy or lower alkanoyloxy, alkenoyloxy, alkynoyloxy, or aryloyloxy; or

I and J together form an oxo; and

K is hydrogen or hydroxy, lower alkoxy, alkanoyloxy, alkenoyloxy, alkynoyloxy, or aryloyloxy

P and Q are independently hydrogen or hydroxy, or lower alkanoyloxy, alkenoyloxy, alkynoyloxy, aryloyloxy,

alkoxycarbonyloxy, aryloxycarbonyloxy or alkylsilyloxy or P and Q together form an oxo; and

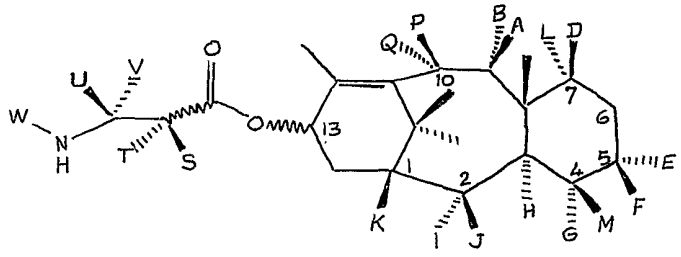
S and T are independently hydrogen, lower alkyl, alkoxy, alkenyl, alkynyl, aryl, aryloxy, substituted aryl or substituted aryloxy; and

U and V are independently hydrogen or lower alkyl, alkenyl, alkynyl, aryl or substituted aryl or heteroaryl and

W and W' are independently hydrogen or lower alkyl, aryl or substituted aryl; and

Y is hydrogen or lower alkyl, aryl or substituted aryl.

The present invention also describes the preparation of taxol intermediates, natural taxol and non-natural occurring taxols of following structural formula:



11

wherein

A and B are independently hydrogen or lower alkanoyloxy, alkenoyloxy, alkynoyloxy, aryloyloxy, or;

A and B together form an oxo;

L and D are independently hydrogen or hydroxy or lower alkanoyloxy, alkenoyloxy, alkynoyloxy, aryloyloxy, alkoxycarbonyloxy, aryloxycarbonyloxy or alkylsilyloxy;

E and F are independently hydrogen or lower alkanoyloxy, alkenoyloxy, alkynoyloxy, aryloyloxy; or E and F together form an oxo;

G is hydrogen or hydroxy or lower alkanoyloxy, alkenoyloxy, alkynoyloxy, or aryloyloxy or;

G and M together form an oxo or methylene or oxirane ring or M and F together form an oxetane ring;
J is hydrogen or hydroxy or lower alkanoyloxy, alkenoyloxy, alkynoyloxy, or aryloyloxy or
I is hydrogen or hydroxy or lower alkanoyloxy, alkenoyloxy, alkynoyloxy, or aryloyloxy; or
I and J together form an oxo; and
K is hydrogen or hydroxy, lower alkoxy, alkanoyloxy, alkenoyloxy, alkynoyloxy, or aryloyloxy
P and Q are independently hydrogen or hydroxy or lower alkanoyloxy, alkenoyloxy, alkynoyloxy, aryloyloxy or alkoxycarbonyloxy, aryloxycarbonyloxy or alkylsilyloxy
P and Q together form an oxo; and
S and T are independently hydroxy, alkoxy, aryloxy, or lower alkanoyloxy, alkenoyloxy, alkynoyloxy, or aryloyloxy; and
U and V are independently hydrogen or lower alkyl, alkenyl, alkynyl, aryl or substituted aryl or heteroaryl and
W is hydrogen, alkanoyl, alkenoyl, alkynoyl, aryloyl, heteroaryloyl, alkoxycarbonyl, aryloxycarbonyl, heteroaryloxycarbonyl.

The taxol alkyl groups either alone or with the variable substituents defined above are preferably lower alkyl containing from one to six carbon atoms in the principal chain and up to 10 carbon atoms. They may be straight or branched chain such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, tert-butyl, pentyl, hexyl and the like.
The taxol alkenyl groups either alone or with the various substituents defined above are preferably lower alkenyl containing from two to six carbon atoms in the principal chain and up to 10 carbon atoms. They may be straight or branched chain and include ethenyl, propenyl, isopropenyl, butenyl, isobutenyl, pentenyl hexenyl, and the like.

The taxol alkynyl groups, either along or with the various substituents defined above are preferably lower alkynyl containing from two to six carbon atoms in the principal chain and up to 10 carbon atoms. They may be straight chain and include ethynyl, propynyl, butynyl, isobutynyl, pentynyl hexynyl and the like.

Exemplary alkanoyloxy include acetate, propionate, butyrate, valarate, isobutyrate and the like, the more preferred alkanoyloxy is acetate.

The taxol aryl moieties either alone or with various substituents contain from 6 to 10 carbon atoms and include phenyl, α- naphthyl etc. Substituents include alkanoxy, hydroxy, halogen, alkyl, aryl, alkenyl, acyl, acyloxy, nitro, amino; amido etc. phenyl is the more preferred aryl.

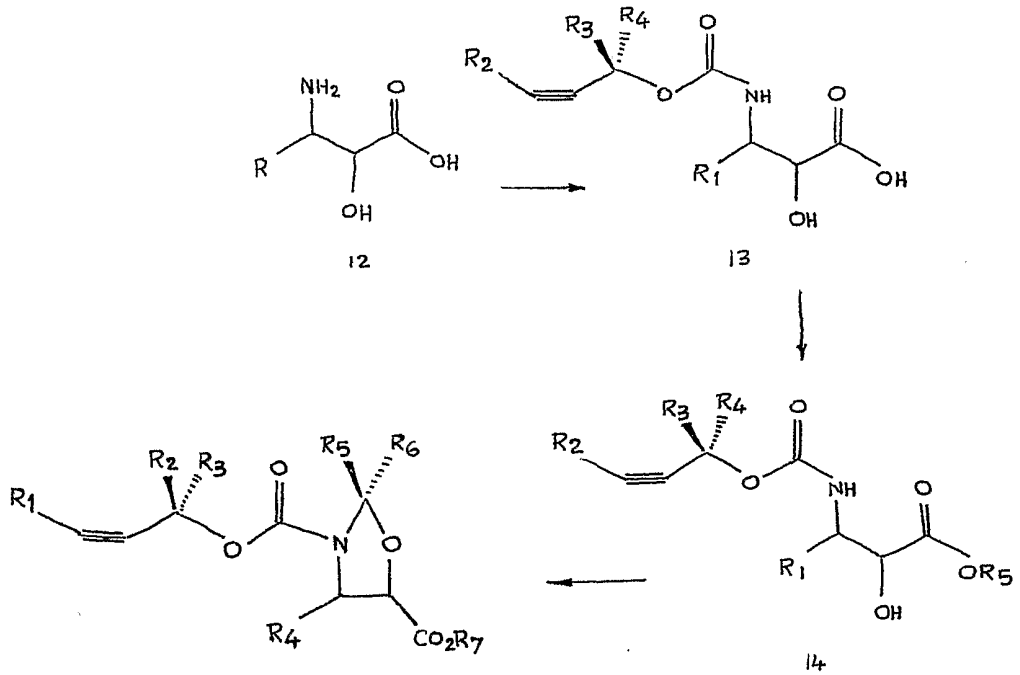
As defined herein, the term aryloyloxy includes aromatic heterocyclic moieties, the term aryl includes any compound having an aromatic ring of which no hetero atom is a member, and the term heteroaryl includes any compound having an aromatic ring which comprises a hetero atom.

Preferred values of the substituents A, B, D, L, E, F, G, M, I, J, K, P, Q, S, T, U, V, W, X and Y are enumerated below in Table-1

A and B together Form an oxo	A=H B=OAc	A=OAc B=H	A=B=H			
D=OH L=H	D=H L=OH	D=OCOOC H_2CCl_3 L=H	D=H L=$OCOOCH_2$ CCl_3	D=OCOR L=H	D=L=H	
E=H F=OAc	E=OAc F=H	E and F together form an oxo	E=H F=O (oxetane)			
G AND M=CH_2	G=CH_2 M=O (Epoxide)	G=O M=CH_2 (Epoxide)	G and M together form an oxo	G = OAc M = CH_2O (Oxetane)	G=H M=CH_2O (Oxetane)	
I=J=O	I=J=H	I=OCOPh J=H	I=OCOR J=H			
K = H	K = OH	K=OCOR	K = OCOR			
P=OH Q=H	P=H Q=OH	P=$OCOCH_2$ CCl_3 Q=H	P=H Q=$OCOCH_2$ CCl_3	P=OCOR Q=H	P=Q=H	
S = T = CH_3	S=H T=Ar	S=H T=OR	S=OR T=H	S=OH T=H	S = H T = OH	
U = Ar V = H	U=H V=Ar					
Structure-10	W=H W'=H	W=CH_3 W'=CH_3	W=H W'=CH_3	W=CH_3 W'=H	W = Ar W'=H	W=H W'=Ar
Strucutre-11	W=H	W=CH_3	W=COAr	W=COOt Bu		
Structure-10	Y=H	Y=CH_3	Y-Ar			

According to this invention, there is provided a process for the preparation of 3-(alk-2-ynyloxy)carbonyl-

5-oxazolidine carboxylic acid. The process is illustrated in reaction 1:



SCHEME - 1

In this process, isoserine 12 is the starting material. In the preferred starting material, R is aryl and the most preferred R is phenyl. The arylisoserine has two asymmetric carbons, all optically active forms such as enantiomers, diastereomers, racemic mixtures and other mixtures are contemplated here. The preferred optically active form of pheneylisoserine is 2R, 3S. This phenylisoserine is commercially available.

Thus, phenylisoserine is condensed with alk-2-ynyl haloformate in presence of some base such as alkali hydroxide or carbonate or bicarbonate or any other acid

neutralising chemical. Herein, alk-2-ynyl groups, either alone or with the various substitutents defined above are preferably lower alk-2-ynyl containing from three to six carbon atoms in the principal chain and up to 10 carbon atoms and include but-2-ynyl, pent-2-ynyl, prop-2 ynyl, hex-2-ynyl and the like.

The most preferred haloformate is prop-2-ynyl chloroformate which is available commercially. This can be prepared from diphosgene/triphosgene and propargyl alcohol following the procedure given in Helv.Chim.Acta. Vol 77, 561, 1994.

The most preferred base here is sodium bicarbonate. The condensation is carried out by addition of chloroformate into a solution of isoserine in aqueous bicarbonate over a period of 5-30 min, most preferably over 10-15 min. The acidification of the reaction mixture affords the N-(alk-2-ynyloxy)carbonylisoserine 13. The most preferred one is where R_1 is phenyl, R_2, R_3, and R_4 are hydrogen.

N-(Alk-2-ynyloxy)carbonylisoserine is then converted into corresponding ester in presence of an alcohol and an activating agent such as dicyclohexylcarbodiimide or carbonyldiimidazole. The most preferred alcohol is methanol and the most preferred activating agents is carbonyldiimidazole. The esterification can be carried out in various solvents such as dichloromethane, chloroform, ethyl acetate, tetrahydrofuran, acetonitrile or dimethyl-formamide. The most preferred solvent is acetonitrile. The esterification is carried out by first mixing acid with activating agent for 0.5-6 hr, most preferably for 1 hr, at 0-25^{o}C, most preferably at 10^{o}C. Then the alcohol is introduced in the mixture over a period of 10-60 min,

most preferably over 30 min and the mixing is done for 1-6 hr, most preferably for 2 hr.

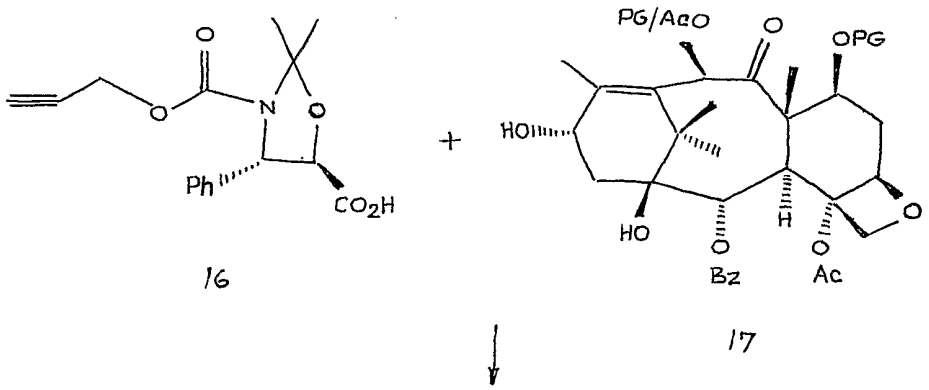
Methyl ester is alternatively prepared by condensing isoserine with diazomethane. The ethereal solution of diazomethane is prepared from following the standard procedure described in Vogel's Text book of Practical Organic Chemistry, 1989, page 432. The methylation is effected by mixing the solution of N-(alk-2-ynyloxy)carbony-lisoserine in tetrahydrofuran with excess ethereal solution of diazomethane. The resultant mixture is stored for 1-6 hr, most preferably for 2 hr at 5-25°C, most preferably at 10°C, for complete reaction.

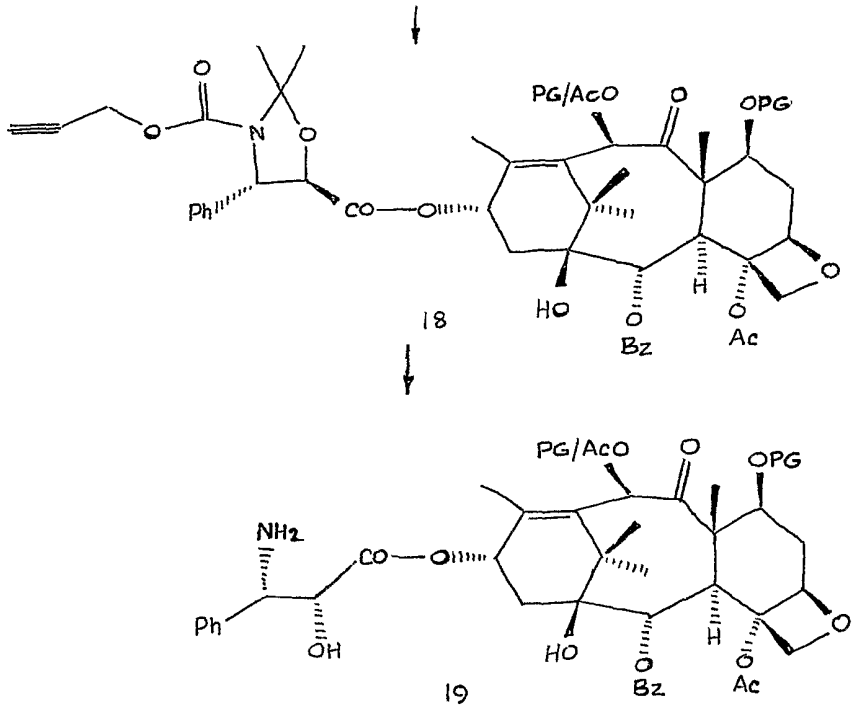
In the most preferred ester 14, R_1 is phenyl, R_2 R_3 and R_4 are hydrogen; and R_5 is methyl.

N-(alk-2-ynyloxy) carbonylisoserine ester 14 is converted into oxazolidine 15 in presence of chemical such as alkoxyalkene or gem-dialkoxyalkane or 1,1,1-trialkoxyalkane. These chemicals are basically reagents for protection of vicinal functional groups such as diols or amino/amidoalchohols in cyclic form. The appropriate chemical is chosen on the basis of type of protecting group required. The most preferred alkoxylalkene, 2-methoxypropene, is available commercially. The reactions are catalysed by p-arylsulfonic acid or their pyridinium salt. The most preferred catalyst is pyridinium p-toluenesulfonate. The protection with 2-methoxypropene results in formation of 3-(alk-2-ynyloxy) carbonyl-5-oxazolidine carboxylic ester 15. In the most preferred oxazolidine ester, R_1, R_2 and R_3 are hydrogen, R_4 is phenyl; R_5, R_6 and R_7 are methyl.

3-(Alk-2-ynyloxy) carbonyl-5-oxazolidine carboxylic ester is finally converted into corresponding acid by hydrolysis with alkali hydroxide or carbonate and mineral acid used successively. The hydrolysis is effected by mixing of a solution of ester in alcohol with an Aq. alkali hydroxide. The most preferred alcohol is methanol and the most preferred alkali hydroxide is sodium hydroxide. The mixing is done for 1 to 6 hr, the most preferably for 3 hr. After the hydrolysis is over, the reaction mixture is acidified to pH 3-6, most preferably 4.5. The acid 16 is isolated by extraction with dichloromethane. In the most preferred acid, R_1, R_2, and R_3 are hydrogen, R_4 is phenyl; R_5 and R_6 are methyl.

There is also provided a process of preparation of taxol and synthetic taxol analogs from 3-(alk-2-ynyloxy) carbonyl-5-oxazolidine carboxylic acids and taxane alcohols, which can be derived from natural or unnatural sources having the following reaction as an example:





PG = Protecting groups

SCHEME-2

The most preferred 3-(alk-2-ynyloxy) carbonyl-5-oxazolidine carboxylic is 3-(prop-2-ynyloxy) carbonyl-2,2-di methyl-4-phenyl-5-oxazolidine carboxylic acid. The preferred tetracyclic taxane alcohols are 7-O-triethylsilylbaccatin, 7-O-(2,2,2-trichloro -ethoxy) carbonylbaccatin, 7,10-di-O-(2,2,2-trichloroethoxy) carbonyl-10-deacetylbaccatin, 7, 10-di-O-benzyloxycarbonyl-10-deacetyl baccatin, 7-O-t-butyloxycarbonylbaccatin, 7, 10-di-O-t-butyloxycarbonyl-10-deacetylbaccatin, 7-O-(prop-2-ynyloxy) carbonyl baccatin, and 7,10-di-O-(prop-2-ynyloxy) carbonyl-10-deacetyl baccatin. The most preferred alcohols are 7-O-(2,2,2-trichloroethoxy) carbonylbaccatin, 7,10-di-O-(2,2,2-trichloroethoxy) carbonyl-10-deacetylbaccatin.

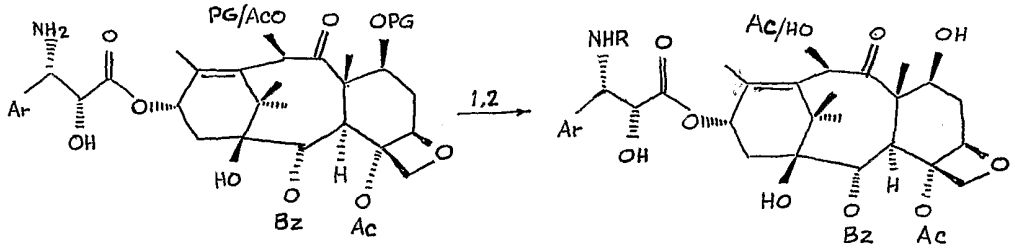
7-O-(2,2,2-trichloroethoxy) carbonylbaccatin, 7,10-di-O-(2,2,2-trichloroethoxy) carbonyl-10-deacetylbaccatin are synthesized from baccatin and 10-deacetylbacctin respectively in presence of 2,2,2-trichloroethoxy

chloroformate and organic base. The preferred bases are pyridine, 4-dimethylaminopyridine or imidazole or like. The most preferred base is pyridine.

The 3-(prop-2-ynyloxy) carbonyl-2, 2-di methyl-4-phenyl-5 oxazolidine carboxylic acid 16 is converted into taxane ester 18 in presence of taxane alcohol such as 7-O-(2,2,2-trichloroethoxy) carbonylbaccatin 17 and condensing agent, preferably dicyclohexylcarbodiimde and organic base preferably 4-dimethylaminopyridine. This process can also be used to prepare various taxane esters 10 contemplated within the present invention from various 3-(alk-2-ynyloxy) carbonyl-5oxazolidine carboxylic acids and taxane alcohols.

The opening of the oxazolidine ring in ester 18 is effected by removal of prop-2-ynyloxy carbonyl with ammonium tetrathiomolybadate preferably benzyltriethyl ammonium tetrathiomolybadate which is prepared according to the known procedure described in Syn.Comm., 22(22), 3277-3284(1992). The removal of prop-2-ynyloxycarbonyl group is effected by exposing the mixture of ester and acetonitrile to ultrasound for 1-6 hr, most preferably for 3 hr. The reaction mixture is diluted with dichloromethane and then washed with water. Removal of the solvents gives the intermediate 19, free amine. The same procedure can be applied to prepare other free amino taxane intermediates 11 (where W is hydrogen) contemplated within the present invention.

The resultant free amine can then be converted into taxol 1 or non-natural taxols such as 2 and 11 contemplated within the present invention following the known methods as illustrated in scheme-3



19 Ar = Ph , PG = $COOCH_2CCl_3$ Taxol Ar = Ph , R = Bz

1. Acylation of Amine
2. Deprotection of Protecting Groups

SCHEME - 3

The invention will now be explained in greater details with the help of the accompanying examples:

Example 1:

(2R, 3S) phenylisoserine (25 gm, 0.138 mole) was dissolved in sodium bicarbonate and potassium bicarbonate (2.5 L) and then 0-5°C. Prop-2-ynyl chloroformate (25 gm, 0.21 mmol) was added drop wise into the solution in a period of 10 to 15 mins. After complete addition the reaction mixture was stirred for 6 Hrs at 05°C. pH of the solution was brought down to 4 by drop wise addition of 10% hydrochloric acid, at temperature 5-6°C. The acidified aqueous layer was extracted with a mixture of tetrahydrofuran and dichloromethane (4:1, 3 x 1L). The combined organic layer was washed with water until the neutral pH.

The organic layer was dried over sodium sulphate and filtered evaporated in under vacuum below 40°C. The residue was dried in vacuum until constant weight to yield 20 g of compound 13.

Example 2:

To a ice cooled solution of Comp-13 (20 g, 76 mmol) in tetrahydrofuran (500 ml) was added drop wise a saturated ethereal solution of diazomethane. The addition was continued until the colour of diazomethane persists. Stirring was continued for further 2 Hrs. The excess diazomethane was removed by drop wise addition of acetic acid. The reaction mixture was then washed with 7% solution of sodium bicarbonate. The organic layer was washed with brine and dried over sodium sulfate. The organic layer was filtered and evaporated. The crude product was purified over silica gel (230-400) (eluent; hexane-ethyl acetate, 7:3 to 1:1) yield comp-14 (18 gm).

Example 3:

To a stirred solution of Comp 13 (5.0 g, 19 mmol) in tetrahydrofuran (50 ml) was added 1,1 carbonyl diimidazole (6.2 gm, 38 mmol) at 0-10°C. The stirring was continued for further 1 Hr. Methanol (3.2 ml) was then added drop wise into the solution in a period of 30 min. After complete addition, the reaction mixture was stirred for 2 Hr, when T.L.C. indicated completion of the reaction. The reaction mixture was concentrated and the residue dissolved in ethyl acetate (2 x 100 ml), washed with 5% hydrochloric acid, water and brine. The organic layer was dried and evaporated. The crude product was chromatograph over silica gel (eluent hexane : ethyl acetate 7:3 to 1:1) yield to comp 14 (4.5 gm).

Example 4:

To a stirred solution of Comp-14 (5g 18 mmol) in toluene (180 ml) was added 2-methoxypropene (35 ml, 0.36 mole), and pyridinium p-toluenesulfonate (425 mg). The mixture was stirred at 25°C for 10-15 min and then heated 80°C for 6 Hr. Progress of the reaction was checked by T.L.C. After completion, the reaction, mixture was cooled and diluted with ethyl acetate (300 ml). The organic layer was washed with 5% sodium bicarbonate (2 x 125 ml) then with brine (150 ml). The organic layer was dried over sodium sulfate. After filtration, the organic layer was evaporated in rotavapour under vacuum below 40°C. The crude product was purified by column chromatography over silica gel, 240-400 mesh (eluent lower 85:15 hexane and ethyl acetate to yield comp-15, (3 g) as a colourless oil.

Example 5:

To a stirred solution of comp-15 (3.5 g, 11 mmol) in methanol (35 ml) was added a solution of lithium hydroxide (1.8 g) in water (15 ml). After stirring at 20-25°C for 3 Hr (absence of starting material, was checked by T.L.C.). The reaction mixture was concentrated to reduce the volume to 15 ml and diluted with dichloromethane (200 ml). The mixture was then washed with water (250 ml). The aqueous layer was separated and cooled to 5-15°C and solution pH was brought to 4 by adding 10% hydrochloric acid. The

aqueous layer was extracted with dichloromethane (3 x 300 ml). The combined organic layer was washed with brine and dried over, preferably sodium sulfate and magnesium sulfate. After filtration the organic layer was evaporated under vaccum and residue was dried up to constant weight to yield compound - 16 (2.2 gm).

Example 6:

To a stirred solution of comp-16 (2.0 g, 6.6 mmol) in toluene or (20 ml) was added dicyclohexylcarbodimide (3g) followed by addition of 7-O-trichloroethoxycarbonylbaccatin 17, (2 g, 2.62 mmol) and DMAP (101 mg). The stirring was continued for 1 Hr. The reaction mixture was diluted with ethyl acetate (100 ml) and filtered. The filtrate was washed successively with aqueous saturated sodium bicarbonate and brine. The organic layer was dried and evaporated under vaccum. The crude product was purified over silica gel (eluent 4:1 hexane and ethyl acetate) to yield comp-18 (1.9 g).

Example-7

A mixture of comp-18 (2 g, 1.91 mmol) and benzyl triethyl-ammonium tetrathiomolybedate (1.16 g) in acetonitrile (5 ml) was kept in ultrasound bath for 4 hr and then diluted with dichloromethane (30 ml). The mixture was washed with water (2x10 mL). The organic layer was evaporated to give comp-19 (1.75 g) as solid.

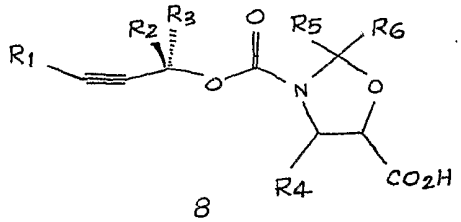
Example-8

A mixture of comp-19 (1 g, 1.08 mmol) in ethyl acetate (5 mL) and saturated Aq. sodium bicarbonate (3 mL) was

added benzoyl chloride (0.17 mL) in ethyl acetate (2 mL) drop wise at 0°C. After, the complete addition, the temperature was raised to 25°C and the stirring was continued for further 2 hr. The Aq. layer was separated and the organic layer was washed with water until neutral. The organic layer was dried over sodium sulfate and then evaporated to give a solid. A mixture of this solid, acetic acid (10 mL), methanol (5 mL) and zinc powder (1 g) was heated to 60°C with stirring. After 1 hr, the reaction mixture was cooled and then filtered. The filtrate was evaporated. The residue was dissolved in ethyl acetate and then washed with Aq. sodium bicarbonate, water and brine. The organic layer was dried over sodium sulfate, then filtered and evaporated. The solid residue was crystallised with acetone-hexane then with methanol- water to afford Taxol (0.74 g, yield 80% from comp-18 99% HPLC w/w assay, 99% HPLC chromatographic purity).

WE CLAIM:

1.3-(alk-2-ynyloxy) carbonyl- 5-oxazolidine carboxylic acid and its analogs having a formula

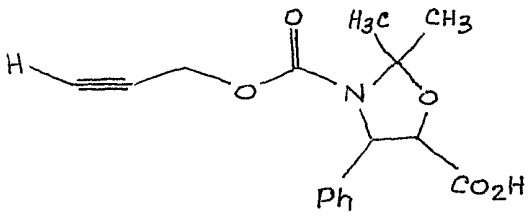


8

and wherein R_1 is hydrogen, aryl, heteroaryl, alkyl, alkenyl, alkynyl, R_2 and R_3 are independently selected from hydrogen, alkyl, alkenyl, alkynyl, aryl, heteroaryl, R_4 is hydrogen, alkyl, alkenyl, alkynyl, aryl, substituted aryl, heteroaryl,

R_5 and R_6 independently selected from hydrogen, alkyl, alkenyl, alkynyl, arly, heteroaryl, alkoxy, alkeyloxy, alkynyloxy, aryloxy, heteroaryloxy.

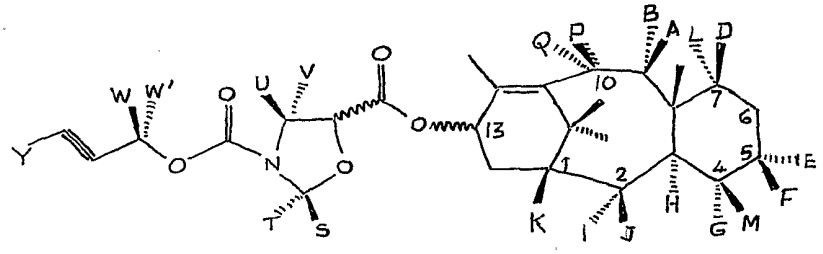
2. Oxazolidine carboxylic acid as claimed in claim 1 having formula wherein R_1 is hydrogen, R_2 and R_3 are hydrogen, R_4 is phenyl and R_5 and R_6 are methyl



9

The present invention also describes process of preparation of 3-(alk-2-ynyloxy)carbonyl- 5-oxazolidine carboxylic acid and its analogs.

3. Taxol intermediates of following structural formula:



10

wherein

A and B are independently hydrogen or lower alkanoyloxy, alkenoyloxy, alkynoyloxy aryloyloxy or;

A and B together form an oxo;

L and D are independently hydrogen or hydroxy or lower alkanoyloxy, alkenoyloxy, alkynoyloxy, aryloyloxy, alkoxycarbonyloxy, aryloxycarbonyloxy or alkylsilyloxy;

E and F are independently hydrogen or lower alkanoyloxy, alkenoyloxy, alkynoyloxy aryloyloxy; or E and F together form an oxo;

G is hydrogen or hydroxy or lower alkanoyloxy, alkenoyloxy, alkynoyloxy, or aryloyloxy or;

G and M together form an oxo or methylene or oxirane ring or

M and F together form an oxetane ring;

J is hydrogen or hydroxy or lower alkanoyloxy, alkenoyloxy, alkynoyloxy, or aryloyloxy or

I is hydrogen or hydroxy or lower alkanoyloxy, alkenoyloxy, alkynoyloxy, or aryloyloxy; or

I and J together form an oxo; and

K is hydrogen or hydroxy, lower alkoxy, alkanoyloxy, alkenoyloxy, alkynoyloxy, or aryloyloxy

P and Q are independently hydrogen or hydroxy or lower alkanoyloxy, alkenoyloxy, alkynoyloxy, aryloyloxy, alkoxycarbonyloxy, aryloxycarbonyloxy or alkylsilyloxy or P and Q together form an oxo; and

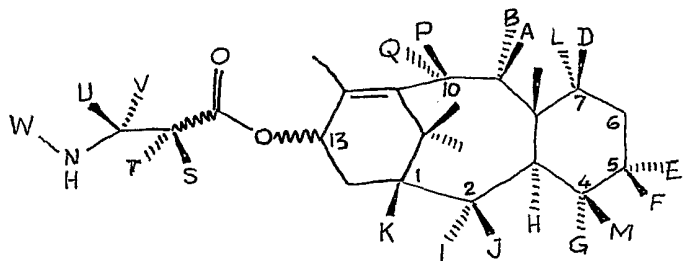
S and T are independently hydrogen, lower alkyl, alkoxy, alkenyl, alkynyl, aryl, aryloxy, substituted aryl or substituted aryloxy; and

U and V are independently hydrogen or lower alkyl, alkenyl, alkynyl, aryl or substituted aryl; and

W and W^1 are independently hydrogen or lower alkyl, aryl or substituted aryl; and

Y is hydrogen or lower alkyl, aryl or substituted aryl.

4. Taxol intermediates natural taxol and non-natural occurring taxols of following structural formula:



11

wherein

A and B are independently hydrogen or lower alkanoyloxy, alkenoyloxy, alkynoyloxy aryloyloxy, or;

A and B together form an oxo;

L and D are independently hydrogen or hydroxy or lower alkanoyloxy, alkenoyloxy, alkynoyloxy, aryloyloxy, alkoxycarbonyloxy, aryloxycarbonyloxy or alkylsilyloxy;

E and F are independently hydrogen or lower alkanoyloxy, alkenoyloxy, alkynoyloxy, aryloyloxy; or E and F together form an oxo;

G is hydrogen or hydroxy or lower alkanoyloxy, alkenoyloxy, alkynoyloxy, or aryloyloxy or;
G and M together form an oxo or methylene or oxirane ring or M and F together form an oxetane ring;
J is hydrogen or hydroxy or lower alkanoyloxy, alkenoyloxy, alkynoyloxy, or aryloyloxy or
I is hydrogen or hydroxy or lower alkanoyloxy, alkenoyloxy, alkynoyloxy, or aryloyloxy; or
I and J together form an oxo; and
K is hydrogen or hydroxy, lower alkoxy, alkanoyloxy, alkenoyloxy, alkynoyloxy, or aryloyloxy
P and Q are independently hydrogen or hydroxy or lower alkanoyloxy, alkenoyloxy, alkynoyloxy, aryloyloxy alkoxycarbonyloxy, aryloxycarbonyloxy or alkylsilyloxy or
and Q together form an oxo; and
S and T are independently hydroxy, alkoxy, aryloxy, or lower alkanoyloxy, alkenoyloxy, alkynoyloxy, or aryloyloxy; and
U and V are independently hydrogen or lower alkyl, alkenyl, alkynyl, aryl or substituted aryl or heteroaryl; and
W is hydrogen, alkanoyl, alkenoyl, alkynoyl, aryloyl, heteroaryloyl, alkoxycarbonyl, aryloxycarbonyl, heteroaryl-oxycarbonyl.

5. Taxol intermediates as claimed in claims 3 and 4 wherein the taxol alkyl groups either alone or with the variable substituents defined above are preferably lower alkyl containing from one to six carbon atoms in the principal chain and up to 10 carbon atoms and comprise straight or branched chain such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, tert-butyl, pentyl, hexyl.

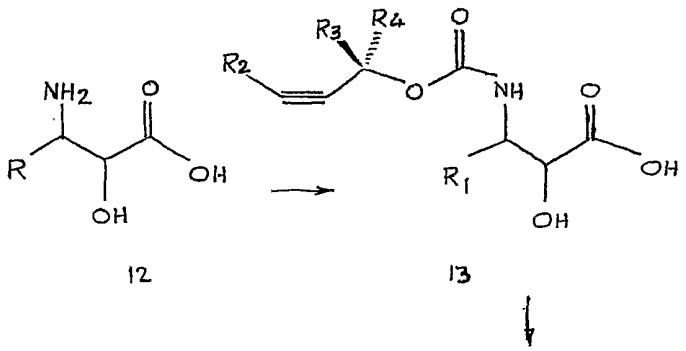
6. Taxol intermediates as claimed in claims 3 and 4 wherein

the taxol alkenyl groups either alone or with the various substituents defined above are preferably lower alkenyl containing from two or six carbon atoms in the principal chain and up to 10 carbon atoms and comprise straight chain and include ethenyl, propenyl, isopropenyl, butenyl, isobutenyl, pentenyl, hexenyl, and the like.

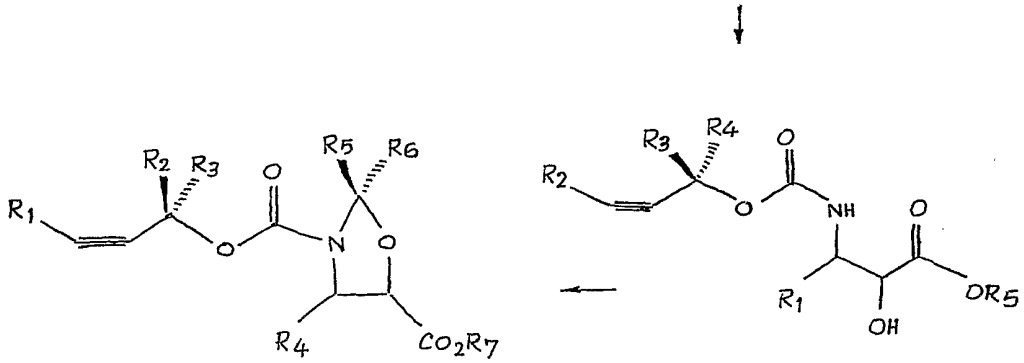
7. Taxol intermediates as claimed in claims 3 and 4 wherein the taxol alkynyl groups, either along or with the various substituents defined above are preferably lower alkynyl containing from two to six carbon atoms in the principal chain and up to 10 carbon atoms and comprise straight chain and include ethynyl, propynyl, butynyl, isobutynyl, pentynyl, hexynyl and the like.

8. Taxol intermediates as claimed in claims 3 and 4 wherein the taxol aryl moieties either alone or with various substituents contain from 6 to 10 carbon atoms and include phenyl, -naphthyl etc.and substituents include alkanoxy, hydroxy, halogen, alkyl, aryl, alkenyl, acyl, acyloxy, nitro, amino, amido etc. phenyl is the more preferred aryl.

9. A process for the preparation of 3-(alk-2-ynyloxy) carbonyl-5-oxazolidine carboxylic acid having the following reaction:



12 13



15 R_7 = Alkyl
16 R_7 = H

14

and wherein isoserine of formula 12 is condensed with alk-2-ynyl haloformate in the presence of a base to produce N-(alk-2-ynyloxy) carbonylisoserine of formula 13, converting said carbonylisoserine into corresponding ester of formula 14 in the presence of alcohol, converting the ester into oxazolidine ester of formular 15, converting said ester by ester hydrolysis to produce the corresponding acid of formula 16.

10. A process as claimed in claim 9 wherein R in formula 12 is aryl, and preferably phenyl, the arylisoserine having two asymmetric carbons, all optically active forms such as enantiomers, diastereomers, racemic mixtures and other mixture. The preferred optically active form of pheneylisoserine being 2R, 3S.

11. A process as claimed in claim 9 wherein the condensation is carried out by addition of chloroformate into a solution of isoserine in aqueous bicarbonate over a period of 5-30 min, most preferably over 10-15 min.

12. A process as claimed in claim 9, wherein the compound

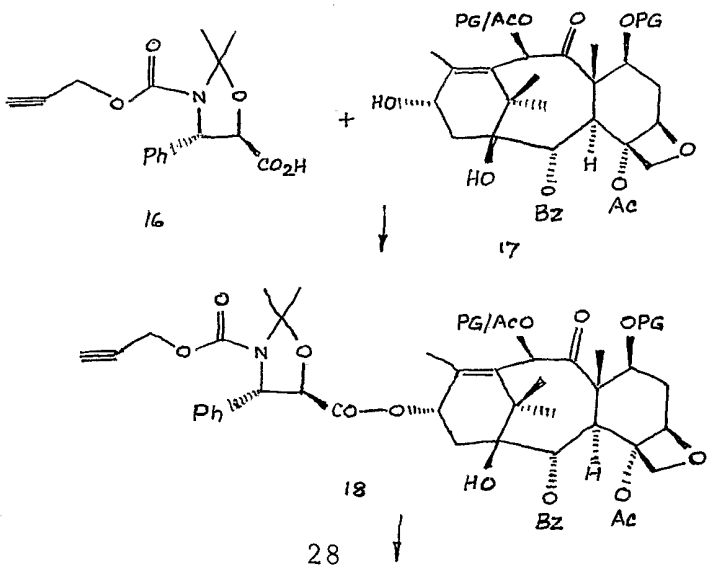
of formula 13 is converted into corresponding ester in presence of an alcohol and an activating agent such as dicyclohexylcarbodiimide or carbonyldiimidazole.

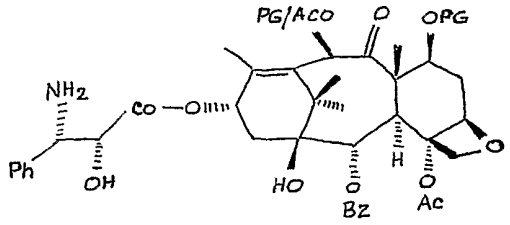
13. A process as claimed in claim 12, wherein esterification is carried out by first mixing acid with activating agent for 0.5-6 hr, most preferably for 1 hr at 0-25°C, most preferably at 10°C; alcohol being introduced in the mixture over a period of 10-60 min, most preferably over 30 min and the mixing is done for 16 hr, most preferably for 2 hr.

14. A process as claimed in claim 9, wherein oxazolidine ester is converted into corresponding acid by ester hydrolysis with alkali hydroxide or carbonate and mineral acid used successively.

15. A process as claimed in claim 14, wherein hydrolysis is effected by mixing of a solution of ester in alcohol with an Aq. alkali hydroxide.

16. A process for preparation of taxol and synthetic taxols having the following reaction:





19

And wherein oxazolidine carboxylic acid of formula 16 is condensed with taxane alcohol of formula 17 using a condensing agent and an organic base to produce the ester of formula 18 which is then treated with tetrathiomolybadate to produce an amine of formula 19. The amine 19 then can be converted into taxol and synthetic taxol analogs using known procedures.

17. A process as claimed in claim 16, wherein the said condensing agent is preferably dicyclohexylcarbodiimde.

18. A process as claimed in claim 16, wherein the said organic base is preferably 4-dimethylaminopyridine.

19. A process as claimed in claim 16, wherein said taxane alcohol is 7-O-(2,2,2-trichloroethoxy) carbonylbaccatin.

20. A process as claimed in claim 16, wherein said oxazolidine carboxylic acid is 3-(prop-2-ynyloxy) carbonyl-2, 2-dimethyl-5-oxazolidine carboxylic acid.

21. A process as claimed in claim 16, wherein said prop-2-ynyloxy carbonyl group cleaving reagent is benzyltriethylammonium tetrathiomolybadate.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IN 01/00020

CLASSIFICATION OF SUBJECT MATTER

IPC[7]: C07D 263/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC[7]: C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO: WPI DATABASE, REGISTRY AND CA DATABASES, STN (Karlsruhe)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5811550 A (DENIS et al.) 22 September 1998 (22.09.98) *column 1-9.*	1,3,5,6,7,8
A	*the whole document.*	2,4,9-21
X	WO 93/16060 A1 (RHONE-POULENC RORER S.A.) 19 August 1993 (19.08.93) *claims.*	1,3,5,6,7,8
A	*the whole document.*	2,4,9-21
X	WO 94/07878 A1 (RHONE-OULENC RORER S.A.) 14 April 1994 (14.04.94) *claims.*	1,3,5,6,7,8
Y	*the whole document.*	2,4,9-21

☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
„A" document defining the general state of the art which is not considered to be of particular relevance
„E" earlier application or patent but published on or after the international filing date
„L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
„O" document referring to an oral disclosure, use, exhibition or other means
„P" document published prior to the international filing date but later than the priority date claimed
„T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
„X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
„Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
„&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 August 2001 (30.08.2001)	6 December 2001 (06.12.2001)
Name and mailing adress of the ISA/AT Austrian Patent Office Kohlmarkt 8-10; A-1014 Vienna Facsimile No. 1/53424/535	Authorized officer SLABY Telephone No. 1/53424/348

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IN 01/00020

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

The passage at page 22 (claim 2): "The present invention also describes process of preperation of 3-alk-2-ynyloxy)carbonyl-5-oxazolikine carboxylic acid and its analogs." does not include substantial feature.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int[illegible]al application No.
PCT/IN 01/00020

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/IN 01/00020

Patent document cited in search report	Publication date	Patent family member(s)			Publication date
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		LV	B	10858	20-06-1996
		MX	A1	9305767	31-05-1994
		NO	A	951202	29-03-1995
		NO	A0	951202	29-03-1995
		NO	B1	310412	02-07-2001
		NZ	A	256446	25-06-1996
		PL	A1	308306	24-07-1995
		PL	B1	179147	31-07-2000
		RU	C1	2116302	27-07-1998
		SG	A1	63619	30-03-1999
		SK	A3	435/95	09-08-1995
		SK	B6	280624	16-05-2000
		US	A	5637723	10-06-1997
		ZA	A	9307319	22-04-1994