

9

PATENT SPECIFICATION

(11) 1 590 649

1 590 649

- (21) Application No. 49906/77 (22) Filed 30 Nov. 1977
 (31) Convention Application No. 747 575
 (32) Filed 6 Dec. 1976 in
 (33) United States of America (US)
 (44) Complete Specification published 3 June 1981
 (51) INT CL³ C07D 519/04; A61K 31/495
 (52) Index at acceptance

C2C 136X 137I 200 211 213 214 215 247 248 250 252 255 258
 25Y 28X 29X 29Y 305 30Y 313 31Y 339 34Y 350 351
 352 360 362 363 366 368 36Y 389 623 627 628 633
 635 638 658 65X 672 678 67X 72Y 761 768 802 80Y
 AA KA TA TX

- (72) Inventors JEAN CORINNE MILLER and GERALD EDWARD GUTOWSKI

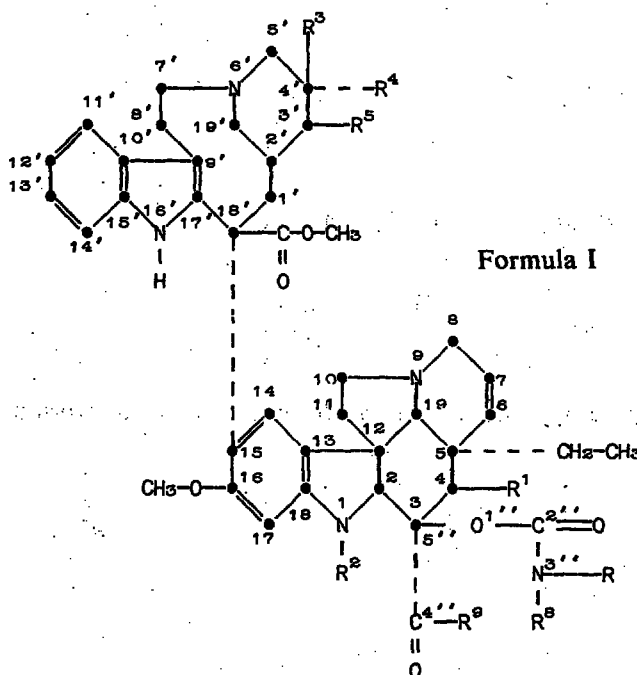


(54) DERIVATIVES OF VINBLASTINE AND RELATED VINCA ALKALOIDS AND THEIR USE IN PHARMACEUTICAL COMPOSITIONS

(71) We, ELI LILLY AND COMPANY, a corporation of the State of Indiana, United States of America, having a principal place of business at 307 East McCarty Street, City of Indianapolis, State of Indiana, United States of America, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to derivatives of Vinca alkaloids, useful as anti-tumor agents and as intermediates, and to a process for their preparation.

The present invention provides a dimeric indoledihydroindole of the formula



wherein

R is H, C₁—C₄ alkyl, C₃—C₄ alkenyl, CH₂—CHX—CH₃ or CH₂—CH₂X wherein

X is Br or Cl;

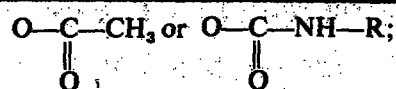
R¹ is OH,

(10)

2

1,590,649

2



R^2 is H, CH_3 or CHO ;

either one of R^3 and R^4 , when taken singly, is H or OH and the other C_2H_5 and R^5 is H;

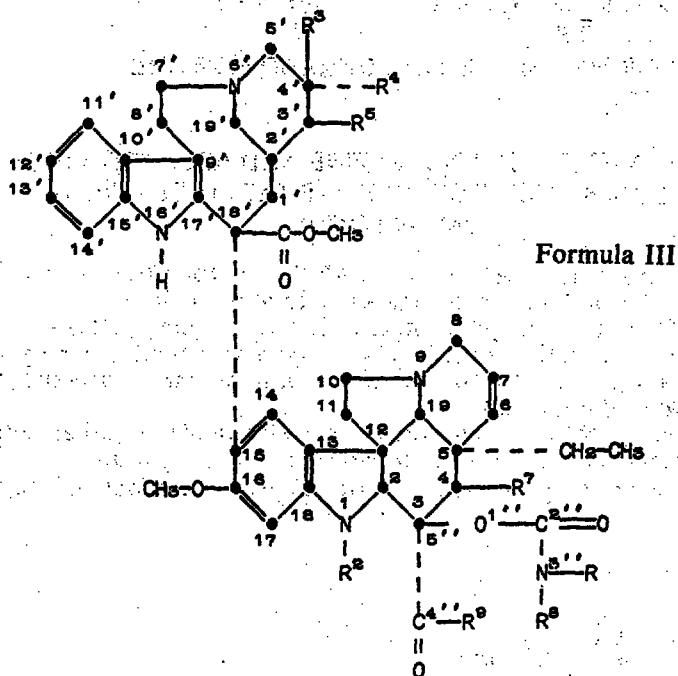
or R^4 and R^5 , taken together, form an epoxide ring and R^3 is C_2H_5 ;

R^6 , taken singly, is H and R^7 , taken singly, is $-\text{O}-\text{CH}_3$;

or R^6 and R^7 , taken together, are a single bond between $\text{C}^{4''}$ and $\text{N}^{3''}$;

and pharmaceutically-acceptable salts thereof.

The invention also provides a process of preparing a dimeric indoledihydro-indole compound of the formula

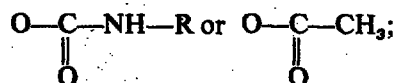


Formula III

wherein

R , R^2 , R^3 , R^4 , R^5 , R^6 and R^8 are as defined above;

R^7 is



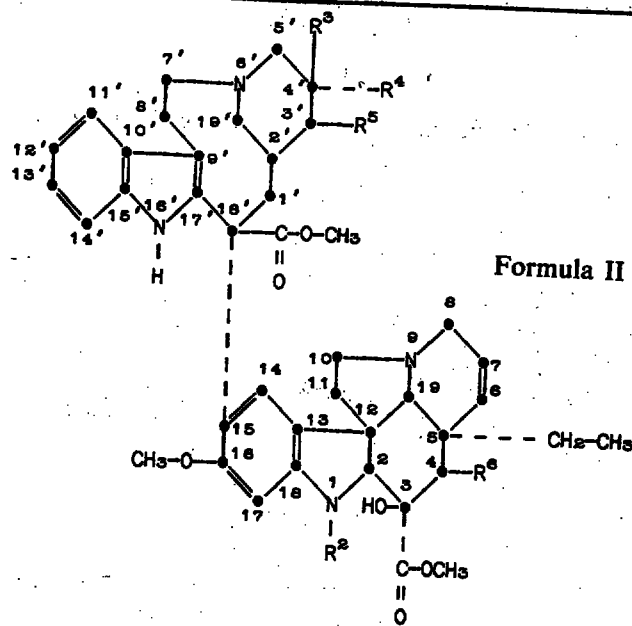
which comprises reacting a dimeric indoledihydroindole of the formula

11

3

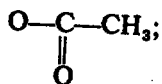
1,590,649

3



wherein

R^2 , R^3 , R^4 , and R^5 have the same meaning as hereinabove; and R^6 is OH or

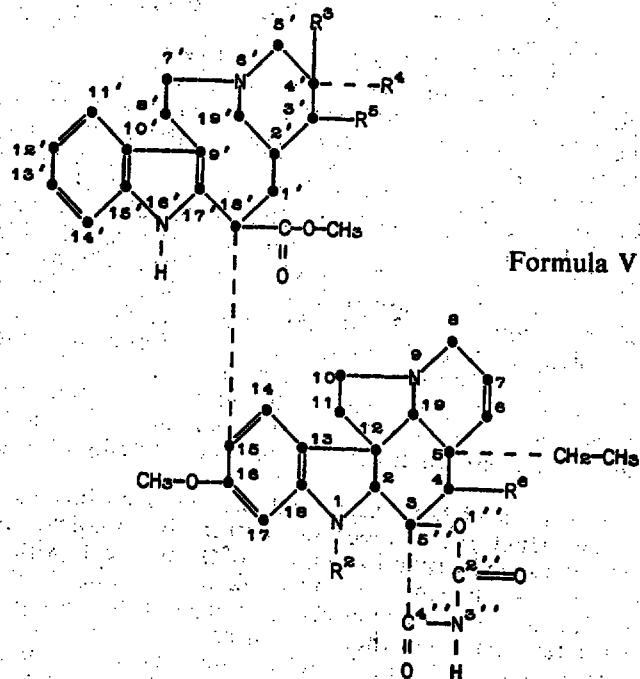


5

5

with an isocyanate of the formula $RNCO$ wherein R has the same meaning as above.

In accordance with the invention there is provided a process for preparing a spirooxazolidinedione of the formula



10

10

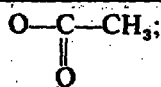
R^2 , R^3 , R^4 and R^5 are as defined above and R^6 is OH or

(12)

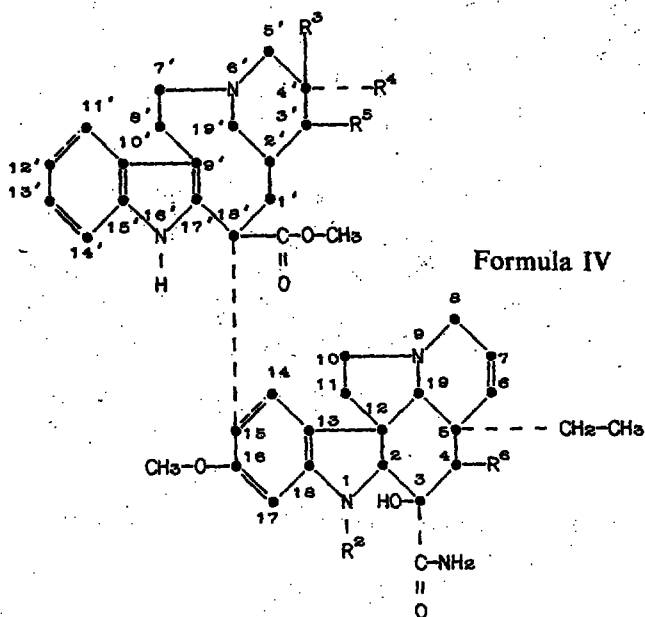
4

1,590,649

4



comprising reacting a dimeric indoleldihydroindole of the formula



wherein

R^2 , R^3 , R^4 , R^5 and R^6 have the same meaning as hereinabove; with dimethylcarbonate in the presence of sodium hydride.

The invention further provides a pharmaceutical composition for the inhibition of tumors or prolongation of the life of the host mammal comprising a carrier and as active ingredient a compound of formula I.

In the above formulae the term " C_1 — C_4 alkyl" includes such alkyl groups as methyl, ethyl, n-propyl, isobutyl, n-butyl, isopropyl, *sec*-butyl, and t-butyl, and the term " C_3 — C_4 alkenyl" includes allyl, methallyl, and crotyl.

Several naturally-occurring alkaloids obtainable from *Vinca rosea* have been found active in the treatment of experimental malignancies in animals. Among these are leurosine (U.S. Patent Specification No. 3,370,057), vincalukoblastine (vinblastine) to be referred to hereinafter as VLB (U.S. Patent Specification No. 3,097,137), leuroformine (Belgian Patent Specification 811,110); leurosidine (vinrosidine) and leurocristine (vincristine) (both in U.S. Patent Specification No. 3,205,220); deoxy VLB "A" and "B", *Tetrahedron Letters*, 783 (1958); 4-desacetoxyvinblastine (U.S. Patent Specification No. 3,954,773; 4-desacetoxy-3'-hydroxyvinblastine (U.S. Patent Specification No. 3,944,554); leurocolombine (U.S. Patent Specification No. 3,890,325) and vincadioline (U.S. Patent Specification No. 3,887,565). Two of these alkaloids, VLB and vincristine, are now marketed as drugs for the treatment of malignancies, particularly the leukemias and related diseases in humans. Vincristine is the more active and useful agent in the treatment of leukemias but is also the less abundant of the anti-neoplastic alkaloids of *Vinca rosea*. These alkaloids are customarily administered by the i.v. route.

Chemical modification of the *Vinca* alkaloids has been limited. In the first place, the molecular structures involved are extremely complex, and chemical reactions which affect a specific functional group of the molecule without changing other groups are difficult to develop. Secondly, alkaloids lacking desirable chemotherapeutic properties have been recovered or produced from *Vinca rosea* fractions or alkaloids, and a determination of their structures has led to the conclusion that these compounds are closely related to the active alkaloids. Anti-neoplastic activity seems to be limited to very specific structures, and the chances of obtaining more active drugs by modification of these structures would seem to be slight. Among the successful modifications of physiologically-active alkaloids has been the pre-

(13)

paration of dihydro VLB (U.S. Patent Specification No. 3,352,868) and the replacement of the acetyl group at C—4 (carbon 4 of the vinca alkaloid ring system—see the numbered structure below) with a higher alkanoyl group or with unrelated acyl groups. (See U.S. Patent Specification No. 3,392,173.) Several of these derivatives are capable of prolonging the life of mice inoculated with P1534 leukemia. One of the derivatives in which a chloracetyl group replaced the C—4 acetyl group of VLB was also a useful intermediate for the preparation of structurally modified VLB compounds in which an N,N-dialkylglycyl group replaced the C—4 acetyl group of VLB (See U.S. Patent Specification No. 3,387,001). C—3 carboxamide derivatives of VLB, vincristine and vincadoline have also been prepared and found to be active anti-tumor agents (Belgian Patent Specification 813,168). These compounds are extremely interesting because, for example, the 3-carboxamides of VLB are more active against Ridgeway osteogenic sarcoma and Gardner lymphosarcoma than is VLB from which they are derived. Certain of the amide derivatives actually approach the activity of vincristine against these tumors. One of these amides, 4-desacetyl VLB C—3 carboxamide or vindesine, is currently on clinical trial in humans and has been found active in certain leukemias. In humans, vindesine appears to have less neurotoxicity than does vincristine.

One of the major medical problems is the treatment and care of neoplastic diseases in humans. There is a variety of these diseases and there is considerable specificity in compounds that have activity against the different diseases. Minor modification in structure of the compound may affect both specificity and activity. These new Vinca derivatives are welcome new weapons in the arsenal of the chemotherapist seeking for new methods of treatment of neoplastic diseases.

Compounds of the above formula I can be described generically as derivatives of VLB where R¹ is acetoxy, R² is methyl, R³ is hydroxyl, R⁴ is ethyl and R⁵ is H, of vincristine where R¹ is acetoxy, R² is formyl, R³ is hydroxyl, R⁴ is ethyl and R⁵ is H, as derivatives of desmethyl VLB (also known as desformylvincristine) where R¹ is acetoxy, R² is hydrogen, R³ and R⁴ are hydroxyl and ethyl, respectively, and R⁵ is H, of leurosine where R¹ is acetoxy, R² is methyl, R³ is ethyl, R⁴ is hydroxyl and R⁵ is H, of Deoxy VLB "A", where R¹ is acetoxy, R² is methyl, R³ and R⁴ are H and R⁵ is ethyl; of Deoxy VLB "B" wherein R¹, R² and R⁵ are the same as in Deoxy VLB "A" but R³ is ethyl and R⁴ is hydrogen, of leurosine wherein R¹ is acetoxy, R² is methyl, R³ is ethyl and R⁴ and R⁵ taken together form an α -epoxide ring or of leuroformine, the corresponding compound in which R²=CHO. The spiro compounds of this invention have been named as 3-spiro-5'-oxazolidinedione derivatives of the particular alkaloid listed above; for example, an oxazolidinedione derived from VLB would be named as 3-descarbomethoxy-3-deshydroxy VLB 3-spiro-5'-oxazolidine-2'',4''-dione. According to the above name, a spiro compound is formed in which the spiro carbon atom is carbon 3 of the vinca alkaloid ring system and carbon 5' of the oxazolidinedione ring system. In naming the compounds of this invention systematically, the terms "3-descarbomethoxy-3-deshydroxy" have been used to indicate that the carbomethoxy group and the hydroxy group at 3 have been replaced by (or incorporated into) the oxazolidine ring. In order to simplify the naming of the compounds of this invention, however, the term "3-descarbomethoxy-3-deshydroxy" will be omitted since the presence of the oxazolidine ring in each of the compounds will be understood to have replaced the hydroxy and carbomethoxy groups at carbon 3 in the vinca alkaloid. It will be understood, therefore, that each name herein of an oxazolidinedione will implicitly contain the terms "3-descarbomethoxy-3-deshydroxy".

Non-toxic acids useful for forming pharmaceutically-acceptable acid addition salts of the compounds of this invention include inorganic acids such as: hydrochloric acid, nitric acid, phosphoric acid, sulfuric acid, hydrobromic acid, hydroiodic acid, nitrous acid and phosphorus acid, as well as salts of non-toxic organic acids including aliphatic mono and dicarboxylic acids, phenyl-substituted alkanic acids, hydroxy alkanic and alkandioic acids, aromatic acids and aliphatic and aromatic sulfonic acids. Such pharmaceutically-acceptable salts thus include the sulfate, pyrosulfate, bisulfate, sulfite, bisulfite, nitrate, phosphate, mono-hydrogenphosphate, dihydrogenphosphate, metaphosphate, pyrophosphate, chloride, bromide, iodide, acetate, propionate, decanoate, caprylate, acrylate, formate, isobutyrate, caprate, heptanoate, propiolate, oxalate, malonate, succinate, suberate, sebacate, fumarate, maleate, butyne-1,4-dioate, hexyne-1,6-dioate, benzoate, chlorobenzoate, methylbenzoate, dinitrobenzoate, hydroxybenzoate, methoxybenzoate, phthalate, terephthalate, benzenesulfonates, toluenesulfonate, chlorobenzenesulfonate, xylenesulfonate, phenylacetate, phenyl-

14

propionate, phenylbutyrate, citrate, lactate, 2-hydroxybutyrate, glycollate, malate, tartrate, methanesulfonate, propanesulfonate, naphthalene-1-sulfonate and naphthalene-2-sulfonate salts.

In the process of this invention a dimeric indoledihydroindole alkaloid having an acetoxyl group at C-4, either obtained by extraction of the leaves of *Vinca rosea* or prepared semi-synthetically by chemical modification of such dimeric compounds or by coupling two monomeric indoles or dihydroindoles, is reacted with an isocyanate RNCO to yield an intermediate 3-carbamate represented by formula III wherein R¹ is H and R² is —O—CH₃. These carbamates are relatively unstable at ordinary reaction temperatures such as 30—110°C and rearrange to the spirooxazolidinediones represented by formula III wherein R¹ and R² are a single bond between C^{4'} and N^{3'}. The stability of the 3-carbamate group depends to a large degree upon the nature of the R-substituent on the carbamate nitrogen. For example, when R is β-chloroethyl, the intermediate 3-carbamate compound can be isolated from the reaction of excess isocyanate and the dimeric alkaloid (II) by chromatography and can be rearranged to a spirooxazolidinedione with heat. 3-Carbamates formed with methylisocyanate, rearrange to the spirooxazolidinedione under standard reaction conditions. By carrying out the reaction at a relatively low temperature the 3-carbamates can be isolated in most cases soon after the isocyanate has been added. Heating for prolonged periods is unnecessary. The isolated intermediate 3-carbamate is rearranged to a spirooxazolidinedione by heating at 30—110°C for several hours.

The 3-carbamates of formula III are not only useful as intermediates in the synthesis of spirooxazolidinediones but also have anti-tumor activity of their own as evidenced by their ability to inhibit the growth of transplanted tumors in mice.

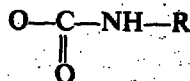
A second type of intermediate is frequently isolated from the reaction of an isocyanate and dimeric indole-dihydroindole alkaloid of Formula II, particularly where an excess of isocyanate is employed and benzene is the solvent. This intermediate is a water-soluble precipitate comprising a complex of the vinca alkaloid and, presumably, more than one molecule of isocyanate per molecule of alkaloid. If, for example, an excess of β-chloroethylisocyanate is reacted with VLB, the subsequent water-soluble complex forms a precipitate which is isolated by filtration. A solution of the filtered complex in water produces the 3'-(β-chloroethyl)spirooxazolidinedione after standing at room temperature for 16 hours. Other isocyanates behave in similar fashion with VLB and other dimeric vinca alkaloids but only certain of the complexes (depending apparently on the nature of the R group of the isocyanate, RNCO) are isolatable at ordinary reaction temperatures.

In carrying out the above Reaction Scheme without isolation of the intermediate carbamate (III), the vinca alkaloid (II) as the free base is dissolved in an inert organic solvent such as benzene and the desired isocyanate (RNCO), usually in excess, is added thereto. The reaction mixture is refluxed until oxazolidinedione formation is substantially complete and is then cooled. Removal of the solvent and excess isocyanate by evaporation yields the oxazolidinedione as a residue which can be further purified as by chromatography. Other inert solvents which can be used in the above reaction include methylene dichloride, chloroform and toluene.

Compounds according to formula I in which R is H are preferably prepared by the following alternative procedure. Vindesine is reacted with dimethylcarbonate in the presence of sodium hydride. The product of this reaction has an unsubstituted spirooxazolidinedione ring but also has an hydroxy in place of an acetoxyl group at C-4 (R¹=OH). Reaction of this compound with 1 mole of acetic anhydride under mild reaction conditions yields a compound according to formula I above wherein R is H and R¹ is acetoxyl.

If a 4-desacetyl alkaloid such as 4-desacetyl VLB or 4-desacetyl vincristine is employed as a starting material, the product of the isocyanate reaction is a 3-spiro-5''-oxazolidine-2'',4''-dione-4-carbamate (O—CO—NHR). These 4-carbamate oxazolidinedione derivatives are also active anti-tumor agents as well as intermediates for the preparation of compounds in which R¹ is OH or, ultimately, acetoxyl.

Compounds with the structure of formula I in which R¹ is OH are prepared by acidic hydrolysis of formula I compounds in which R¹ is acetoxyl e.g. with hydrochloric acid. These compounds in which R¹ is OH can be reacted with an isocyanate (RNCO) to yield compounds in which R¹ is

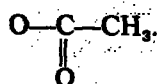


by simple reaction in an inert solvent, preferably with gentle heating. These 4-carbamates are also prepared by utilizing as a starting material in the above reaction scheme a compound in which R⁶ is OH:

Pharmaceutically-acceptable acid addition salts of the 3-spiro-5"-oxazolidine-2",4"-dione derivatives of those vinca alkaloids as represented by formula I are prepared by mixing a solution of the alkaloidal free base with 1 mole of the desired non-toxic acid in an inert solvent. If the salt is soluble, it is recovered by evaporation of the solvent in vacuo. If the salt is insoluble in the reaction medium, it will precipitate and be collected by filtration. Certain of the inorganic salts are prepared by a somewhat different procedure. For example, the sulfate salt is prepared by dissolving a free base according to formula I above in a minimal amount of a polar organic solvent such as ethanol and then adjusting the pH of this solution to about 4.0 by the dropwise addition of 2% aqueous sulfuric acid in ethanol. The sulfate salt is recovered by evaporation of the solvents to dryness. The hydrochloride salt can be prepared in similar fashion by adding an alcoholic solution of HCl to an alcoholic solution of the free base. The acid addition salts prepared as above can be purified by such well-known techniques such as chromatography or recrystallization.

As previously mentioned, the compounds of this invention are also useful as intermediates for the preparation of other anti-tumor vinca alkaloids. Treatment of the oxazolidinedione (I) with base yields a C-3 carboxamide derivative usually lacking the acetyl group at C-4. For example, reaction of VLB 3"-methyl-3-spiro-5"-oxazolidine-2",4"-dione with aqueous alkali yields 4-desacetyl VLB C-3 N-methylcarboxamide. The C-3 carboxamide derivatives of the vinca alkaloids such as VLB, vincristine, leurosine, and deoxy VLB "A" and "B" are active anti-tumor agents in animals. The C-3 carboxamides of leurosine and of leuroformine, which alkaloids are included in the scope of formula II therein, are also active anti-tumor agents against transplanted tumors in experimental animals.

The starting materials according to formula II above are either isolated from the leaves of the plant *Vinca rosea* such as VLB, vincristine, leurosine, leuroformine, deoxy VLB "A" and "B", or, when R⁶ in II is OH, prepared by acidic hydrolysis of the corresponding compound in which R⁶ is



The 4-desacetyl compound can be reacylated if desired at C-4 by the procedure of Hargrove, *Lloydia*, 27, 340 (1964) or by acetylation under mild conditions using 1 mole of acetic anhydride. Starting materials represented by formula II when R² is H or formyl, except for vincristine, N-desformyl leurocristine, N-desformyl-leuroformine and leuroformine, all of which are obtained from leaves of *Vinca rosea*, are prepared as follows. The 1-methyl group of Deoxy VLB "A" or "B", etc. can be oxidized with chromium oxide in glacial acetic acid at -60°C to yield a mixture of compounds in which R² is H or formyl according to the procedure set forth in U.S. Patent Specification 3,899,493. The compounds in which R² is H can be formylated or compounds in which R² is formyl can be deformylated.

This invention is further illustrated by the following specific examples.

Example 1.

Preparation of VLB 3"-methyl-3-spiro-5"-oxazolidine-2",4"-dione.

A solution was prepared containing 2.84 g. of VLB free base in 40 ml. of anhydrous benzene. 15 ml. of methylisocyanate were added thereto and the resulting mixture heated to refluxing temperature for about 6 hours. The reaction mixture was cooled and the volatile constituents removed by evaporation. The residue comprising VLB 3"-methyl-3-spiro-5"-oxazolidine-2",4"-dione formed in the above reaction was purified by chromatography over 200 g. of silica (activity I). The chromatogram was developed with 1 liter of 4% methanol in benzene followed by 2.5 liters of 6% methanol in benzene which eluted VLB 3"-methyl-3-spiro-5"-oxazolidine-2",4"-dione; weight = .9764 g.; molecular ion weight at 835; nmr δ at 0.17, .88, 2.0, 2.60, 2.74, 2.78, 2.81, 3.08 (new NCH₃) 3.58, 3.60, 3.79, 4.52, 5.18, 5.41,

(16)

1,590,649

8

8

5.88, 6.09, 6.64; mass spectrum ion peaks at 849 (trans-methylation), 835, 817, 804, 776, 650, 543, 494, 355, 167, 154, 149, 135.

Using the above procedure 270 mg. of VLB free base was reacted with n-butyl isocyanate to form VLB 3''-n-butyl-3-spiro-5''-oxazolidine-2'',4''-dione (41 mg. purified material). Mass spectrum ion peaks at 891, 877, 859, 837, 836, 819, 818, 792, 747, 650, 543, 536, (512), 381, 380, 355, 341, 325, 295, (188), 154, 149, 135 and 6, 122, 121, 107. Nmr in deuteriochloroform, δ at .66, .92, 1.3—1.7, 1.99, 2.07, 2.81, 3.61, 3.79, 5.17, 5.43, 5.87, 6.09, 6.61.

98.9 Mg. VLB free base was reacted with allyl isocyanate to form VLB 3''-allyl-3-spiro-5''-oxazolidine-2'',4''-dione (31.3 mg. purified material). Mass spectrum ion peaks at 875, 861, 843 and 4, 830, and 1, 802 and 3, (784), 650, 543, 520, 380 and 1, 355, 295, 273, 242, 154, 149, 143, 136, 135, 122, 121, 107. Infrared spectrum in chloroform; peaks at 3440, 1810, 1740, 1610 cm^{-1} . Nmr, δ at .66, .88, 1.98, 2.41, 2.80, 3.57, 3.60, 3.79, 4.14, 5.1—5.3, 5.43, 5.7—5.95, 6.08, 6.63.

Example 2.

Preparation of vincristine 3''-methyl-3-spiro-5''-oxazolidine-2'',4''-dione.

Vincristine free base obtained from 158.3 mg. of vincristine sulfate by standard procedures was dissolved in about 15 ml. of anhydrous benzene. About 2.5 ml. of methylisocyanate were added to the vincristine solution and the resulting mixture was heated to refluxing temperature for about 6 hours and then kept at ambient temperature overnight, followed by an additional 4 hour reflux. The volatile constituents were removed leaving a residue weighing 159.6 mg. which was soluble in methanol but not soluble in methylene dichloride. Preparative thin-layer chromatography using a 3:1 ethyl acetate-ethanol solvent system yielded 4 bands of which the third, in order of polarity, was vincristine 3''-methyl-3-spiro-5''-oxazolidine-2'',4''-dione (26.3 mg. purified material), having the following physical characteristics. Mass spectrum ion peaks at 863, 849, 831, 818, 806, 790, 751, 708, 647, 650, 635, 480, 393, 369, 355, 283, 270, 268, 183, 171, 168, 154, 141, 136, 122, 121. Infrared spectrum in chloroform; peaks at 3440, 1805, 1730, (and 1745), 1660 cm^{-1} . Nmr in deuteriochloroform; δ at .59, .88, 1.35, 1.68, 2.74, 2.78, 3.01, 3.65, 3.87, 4.41, 4.68, 5.20, 5.90, 6.81, 6.91, 8.12, 8.78.

Example 3.

Preparation of 1-desmethyl-4-desacetyl-4-(N-methylcarbamoyloxy) VLB 3''-methyl-3-spiro-5''-oxazolidine-2'',4''-dione.

Following the procedure of Example 1, 21.7 mg. of 1-desmethyl-4-desacetyl VLB were reacted with 3 ml. of methylisocyanate in about 12 ml. of anhydrous methylene dichloride at a refluxing temperature. 1-Desmethyl-4-desacetyl-4-(N-methylcarbamoyloxy) VLB 3''-methyl-3-spiro-5''-oxazolidine-2'',4''-dione formed in the above reaction was isolated by the procedure of Example 1 and purified by preparative thin-layer chromatography using a methanol solvent system (5.5 mg. purified product). The compound had the following physical characteristics. Nmr (in deuteriochloroform); δ at 2.75, 2.79, 3.08, 3.59, 3.76, 3.84, 4.76, 5.35, 5.19, 5.86, 6.26, 6.68. Infrared spectrum in chloroform showed a new peak at 1818 cm^{-1} , and increased absorption at 1740 cm^{-1} and at 1130 cm^{-1} compared with starting material. Mass spectrum ion peaks at 836, 834, 779, 777, 368, 256, 241, 213, 155, and 149.

Example 4.

Preparation of VLB 3''-(β -chloroethyl)-3-spiro-5''-oxazolidine-2'',4''-dione.

Following the procedure of Example 1, 1.620 g. VLB was reacted with 15 ml. of β -chloroethylisocyanate in 100 ml. of anhydrous benzene. The reaction mixture was stirred for 16 hours at room temperature followed by 2 hours heating at reflux temperature. A precipitate comprising a water-soluble complex of VLB and β -chloroethylisocyanate separated and was recovered from the cooled solution by filtration. The precipitate (1.692 g.) was dissolved in about 30 ml. of water and the aqueous solution stirred at room temperature for about 16 hours. The solution was then made basic by the addition of dilute sodium hydroxide and VLB 3''-(β -chloroethyl)-3-spiro-5''-oxazolidine-2'',4''-dione, formed in the above reaction, being insoluble in basic solution, separated and was extracted into methylene dichloride. The methylene dichloride extracts were combined and the solvent removed therefrom by evaporation. The residue comprising VLB 3''-(β -chloroethyl)-3-spiro-5''-oxazolidine-2'',4''-dione was purified by chromatography over 150 g. of silica (activity I) (1.127 g. purified material). The chromatogram was developed with 6%

methanol in benzene. The compound had the following physical characteristics; mass spectrum ion peaks at 883, 847, 816, 789, 650, 592, 591, 543, 506, 485, 451, 355, 295, 154, 136, 135, 122, 121, 107. Infrared spectrum (in chloroform) peaks at 3650, 3570, 3440, 1805, 1745, 1610 cm^{-1} . Nmr in deuterochloroform, δ at .67, .88, 2.01, 2.81, 285, 3.61, 3.79, 3.87, 5.19, 5.44, 5.89, 6.11, and 6.65.

Example 5.

Alternate preparation of VLB 3''-(β -chloroethyl)-3-spiro-5''-oxazolidine-2'',4''-dione and of VLB 3-N-(β -chloroethyl)-carbamate.

A solution was prepared containing 481.2 mg. of VLB free base in 14 ml. of anhydrous benzene. 7.0 ml. of β -chloroethylisocyanate were added thereto. There was an immediate precipitate. The reaction mixture was heated at refluxing temperature for about 16 hours. The benzene was removed by evaporation in vacuo and the residue subjected to preparative thin-layer chromatography over silica using a 3:1 ethyl acetate-methanol solvent system. Two inseparable mobile bands were isolated together (with a total yield of 379.4 mg.) and rechromatographed. A second chromatography using the same 3:1 ethyl acetate-methanol solvent system with only 47 mg. of the 2 component mixture per preparative plate resulted in a separation into VLB 3''-(β -chloroethyl)-3-spiro-5''-oxazolidine-2'',4''-dione (147 mg.) and VLB 3-N-(β -chloroethyl)carbamate (69 mg.). This latter compound had the following physical characteristics; mass spectrum ion peaks at (881), 835, 821, 790, 763, 692, 591, 543, 480, 409, 353, 295, 293, 283, 281, 278, 243, 188, 154, 149, 135. Infrared spectrum in chloroform showed maxima at 3645, 3575, 3450, 3405 (new) 1730, 1675 (new), 1610 cm^{-1} . Nmr in deuterochloroform; δ at .88, .80, 1.35, 2.05, 2.76, 2.80, 3.60, 3.77, 5.32, 5.53, 5.84, 6.09, 6.60, 10.0 (broad).

Example 6.

Preparation of 4-desacetyl VLB 3''-methyl-3-spiro-5''-oxazolidine-2'',4''-dione. 99.7 Mg. of VLB 3''-methyl-3-spiro-5''-oxazolidine-2'',4''-dione were refluxed in 0.5 N aqueous hydrochloric acid for about 1.5 hours. The aqueous solution was made basic and 4-desacetyl VLB 3''-methyl-3-spiro-5''-oxazolidine-2'',4''-dione, being insoluble in base; separated and was extracted into methylene dichloride. The methylene dichloride extracts were combined and the solvents evaporated therefrom in vacuo. The residue was subjected to preparative thin-layer chromatography over silica gel using a 3:1 ethyl acetate-methanol solvent mixture to develop the chromatogram. The main band comprised 4-desacetyl VLB 3''-methyl-3-spiro-5''-oxazolidine-2'',4''-dione (32.3 mg. purified material). The compound was single spot material in three other chromatographic systems. It had the following physical characteristics: mass spectrum ion peaks at 807, 793, 763, 762, 749, 734, 690, 493, 452, 408, 355, 295, 268, 167, 171, 154, 149, 143, 135, 122, 121, 107. Infrared spectrum in chloroform, maxima at 3580, 3440, 1815, 1735, 1615 cm^{-1} . Nmr in deuterochloroform; δ at .80, .88, 2.70, 2.81, 2.90, 3.04, 3.59, 3.76, 3.92, 4.20, 5.60, 5.88.

Example 7.

Preparation of 4-desacetyl-4-N-methylcarbamoyl-VLB 3''-methyl-3-spiro-5''-oxazolidine-2'',4''-dione.

A solution was prepared containing 304 mg. of 4-desacetyl VLB in 10 ml. of anhydrous benzene. 5 ml. of methylisocyanate were added thereto and the resulting solution refluxed for about 6 hours. The solvent was removed by evaporation in vacuo to leave a residue weighing 444.4 mg. Thin layer chromatography indicated that 2 materials were present in the residue and these were separated by preparative thin-layer chromatography using a 3:1 ethyl acetate-methanol solvent system. This procedure yielded purified 4-desacetyl-4-N-methylcarbamoyl-VLB 3''-methyl-3-spiro-5''-oxazolidine-2'',4''-dione having the following physical characteristics: Mass spectrum ion peaks at 850, 807, 793, 775, 762, 734, 452, 355, 154, and 135. Ultraviolet spectrum in ethanol; peaks at 215 and 268 with shoulders at 290 and 298 microns. Infrared spectrum (in chloroform); maxima at 3700, 3460, 1816, 1743, 1600 cm^{-1} . Nmr in deuterochloroform; δ at .67, .88, 2.78, 2.81, 3.07, 3.60, 3.70, 5.0, 5.25, 5.30, 5.84, 6.08 and 6.03. This compound showed metaphase arrest activity in a standard mitotic inhibition test at concentrations as low as 10^{-3} mmg/ml.

18

10

1,590,649

10

Example 8.

Preparation of 4-desacetyl VLB 3''- β -chloroethyl-3-spiro-5''-oxazolidine-2'',4''-dione.

25 ml. of dry methanol saturated with hydrogen chloride at 0°C. was added to 96.9 mg. of VLB 3''- β -chloroethyl-3-spiro-5''-oxazolidine-2'',4''-dione. The mixture was allowed to warm to room temperature and stirred for 18 hours. The solvent was evaporated therefrom in vacuo. Water was added to the residue along with ice. The solution was made basic with ammonium hydroxide and extracted four times with methylene dichloride. The methylene dichloride extracts were combined and the solvents evaporated therefrom in vacuo. The residue (88.6 mg.) was subjected to preparative thin-layer chromatography over silica gel using a 3:1 ethyl acetate-methanol solvent mixture to develop the chromatogram. The band running $r_f = .4$ was eluted with a yield of 14.3 mg. Physical characteristics: mass spectrum peaks at: 841 (parent ion), 816, 782, 651, 543, 355, 295, 175, 167, 154, 149, 135, 125.

Example 9.

Alternate preparation of 4-desacetyl VLB 3''- β -chloroethyl-3-spiro-5''-oxazolidine-2'',4''-dione.

25 ml. of β -chloroethyl isocyanate was added to 9.86 g. of VLB free base in 200 ml. dry benzene. The reaction mixture was stirred overnight at room temperature and then refluxed for 2.5 hours. The precipitate was filtered. The 3.15 g. filtrate was dissolved in 300 ml. 0.1N aqueous hydrochloric acid and refluxed overnight. The reaction solution was cooled, and made basic with ammonium hydroxide and extracted with methylene dichloride. The extracts were combined and the solvent evaporated therefrom in vacuo leaving a residue of 2.80 g. The residue comprising 4-desacetyl VLB 3''- β -chloroethyl-3-spiro-5''-oxazolidine-2'',4''-dione was purified by chromatography over silica gel. This chromatogram was developed with benzene with an increasing methanol gradient. Yield was 192.0 mg. Physical characteristics: Mass spectrum ion peaks were identical to those in Example 8. Nmr in deuteriochloroform, δ at .81, .88, 2.25, 2.71, 2.99, 3.60, 3.76, 3.79, 3.93, 5.61, 5.88, 6.00, and 6.63.

Example 10.

Preparation of VLB 3-spiro-5''-oxazolidine-2'',4''-dione.

A suspension of 208.0 mg. of sodium hydride (as a 50% oil dispersion) was prepared in 20 ml. of tetrahydrofuran. 200.9 mg. of vindesine (VLB C-3 carboxamide) were added thereto. After the solution had been stirred at ambient temperature for 25 minutes, 4.0 ml. of dimethylcarbonate were added. The reaction mixture was then stirred at ambient temperature for 4.5 hours after which time the volatile constituents were removed by evaporation. Water was added and the aqueous solution acidified with dilute hydrochloric acid. The acidic layer was extracted three times with methylene dichloride and the methylene dichloride extracts were discarded. The aqueous layer was then made basic with 10% percent aqueous sodium hydroxide. VLB 3-spiro-5''-oxazolidine-2'',4''-dione, being insoluble in the basic layer separated and was extracted with 4 portions of methylene dichloride. The methylene dichloride extracts were combined and the solvent removed by evaporation. The residue weighing 98.4 mg. was subjected to preparative thin-layer chromatography over silica using a 1:1 ethyl acetate-methanol solvent system. 4 bands were seen, the fourth band comprising 4-desacetyl VLB 3-spiro-5''-oxazolidine-2'',4''-dione. The band was separated mechanically and eluted from the silica. Evaporation of the eluting solvent yielded a residue weighing 10.9 mg. with the following physical characteristics. Nmr in deuteriochloroform; δ at .90, 2.87, 3.57, 3.65, 3.84, 3.95, 5.5-6.0, 6.08, 8.5. Infrared spectrum, maxima at 3680, 3470, 1810, 1735, 1620, 1505, 1460, 1435, 1335, 1010, 910 cm^{-1} . Molecular spectrum; ions at 807, 793, 763, 749, 718, 706, 692, 690, 634, 434, 422, 408, 355, 351, 325, 323, 297, 295, 269, 268, 187, 167, 154, 149, and 135. Field desorption molecular ions; 779, 753, 735.

Oxazolidinediones lacking a substituent on the ring nitrogen can exist in tautomeric forms in which the hydrogen on the ring nitrogen can enolize with either of the carbonyl groups present in the ring to form an hydroxy oxazolinone. More specifically, an oxazolidine-2,4-dione can tautomerize to either a 2-hydroxy-2-oxazoline-4-one or a 4-hydroxy-3-oxazoline-2-one. It is believed that the product of the above reaction contains at least two of such tautomeric forms if not all three.

(19)

Example 11.

Preparation of salts.

Salts of the oxazolidinediones and of the 3-carbamates are prepared as follows:
The free base is dissolved in a minimal quantity of ethanol. The pH is lowered to 4.0 ± 0.2 by dropwise addition of 2 percent sulfuric acid in ethanol. The pH is determined by taking a 2-drop aliquot, diluting the drop to 1 ml. with water and then determining the pH using a pH meter. The acidic ethanolic solution is evaporated to dryness. The residue comprising the sulfate salt can be recrystallized from methanol or ethanol if desired.

The compounds of this invention have been shown to be active against transplanted tumors in mice in vivo and to induce metaphase arrest in Chinese hamster ovary cells maintained in tissue culture. In demonstrating activity of the drugs of this invention against transplanted tumors in mice, a protocol was used which involved the administration of the drug, usually by the intraperitoneal route, at a given dose level for 7—10 days after innoculation with the tumor.

The following table — Table 1 — gives the results of several experiments in which mice bearing transplanted tumors were treated successfully with a compound of this invention. In the table, column 1 gives the name of the compound; column 2, the transplanted tumor; column 3, the dose level or dose level range and the number of days the dosage was administered; column 4, the route of administration, and column 5, the percent inhibition of tumor growth or percent prolongation of survival time, e.g., B16 and P388. (ROS is an abbreviation for Ridgeway osteogenic sarcoma; GLS for Gardner lymphosarcoma; P1534(J) and P388 are leukemias; CA 755 is an adenocarcinoma; and B16 is a melamoma).

TABLE 1

Compound	Tumor	Dose mg./kg. × days	Route	Percent Inhibition or Prolongation of Survival Time
VLB 3''-methyl-3-spiro-5''- oxazolidine-2''-4''-dione free base	GLS	3-5 × 10	PO	37-56%
		0.6 × 7	IP	100%
		1.25 × 9	IP	100%
	L1210	1.0-1.5 × 3	IP	96-102% (4 indefinite survivors)*
Sulfate salt	B16	0.5-0.6 × 10	IP	156-8% (3 indefinite survivors)*
		4.5 × 10	IP	49-91%
	CA755	0.5-1.0 × 10	IP	43-100%
	P1534(J)	5-6 × 10	IP	100%
		3-4 × 8	PO	100%
	GLS ROS	3-4 × 10 5-7 × 10	PO PO	100% 38-59%
4-(N-methylcarbamoyl)- 4-desacetyl VLB 3''-methyl- 3-spiro-5''-oxazolidine- 2''-4''-dione	GLS	0.1-2.5 × 7-9	IP	40-100%
	GLS	0.5-0.6 × 9-10 3-4 × 9	IP PO	50-74% 92-100%

VLB 3''-(β-chloroethyl)-
3-spiro-5''-oxazolidine-
2''-4''-dione
free base

(21)

TABLE 1 (Continued)

Compound	Tumor	Dose mg./kg. x days	Route	Percent Inhibition or Prolongation of Survival Time
Sulfate salt				
	GLS	0.5-0.65 x 10	IP	47-55%
	CA755	0.75-1.0 x 10	IP	53%
	3-5 x 9	3-5 x 9-10	PO	59-92%
	B16	0.4-0.6 x 10	IP	25-178% (5 indefinite survivors)*
	P1534(J)	3.5 x 8	PO	100%
	L1210	0.4-0.6 x 10	IP	61-86%
		1.0-2.0 x 3	IP	53-101% (2-7 indefinite survivors)*
VLB 3-Spiro-5'-oxazolidine- 2'',4''-dione	B16	2-.8 x 10	IP	62-92% (0-3 indefinite survivors)*
4-Desacetyl VLB 3''-methyl-3- spiro-5''-oxazolidine-2'',4''-dione	GLS	2.5 x 7	IP	27%
VLB 3''-allyl-3-spiro-5''- oxazolidine-2'',4''-dione	GLS	2.5 x 7	IP	35%
Vincristine 3''-methyl-3- spiro-5''-oxazolidine-2'',4''- dione	GLS	1.0 x 7	IP	61%
4-Desacetyl VLB 3''-β- chloroethyl-3-spiro-5''- oxazolidine-2'',4''-dione	GLS	0.5-1.0 x 10	IP	27-51%

*Survived for 60 days till end of trial.

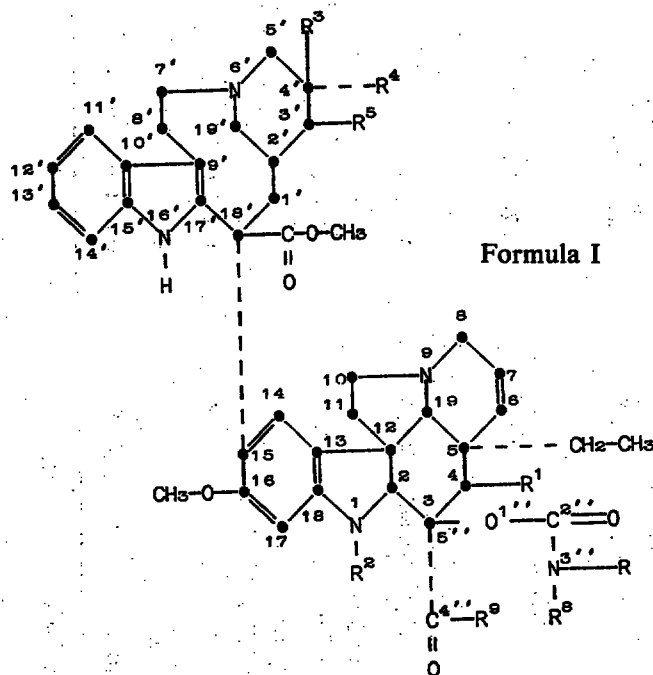
As seen from the above Table, the compounds of this invention are active by the oral route. This oral activity is quite unexpected since the marketed drugs VLB and vincristine do not show this degree of oral activity against the same tumors in experimental animals. For example, VLB 3''-methyl-3-spiro-5''-oxazolidine-2'',4''-dione and its 3''-(β-chloroethyl) analogue give 100% inhibitory activity orally against GLS, ROS and CA755 at dose levels of 6-7 and 3-6 mg./kg. whereas VLB and vincristine are only marginally active, if at all, at 2 and 2-4.5 mg./kg. dose levels respectively and usually are toxic at higher levels. Parenterally, however, (via

the intraperitoneal route) comparable tumor inhibition or prolongation of life is shown at the 0.5—0.75 mg./kg. dose levels for oxazolidine diones and at dose levels of 0.4—0.45 mg./kg for VLB and 0.2—0.25 for vincristine.

In utilizing the novel compounds of this invention as anti-tumor agents in mammals, either the parenteral or oral route of administration may be employed. For oral dosage, a suitable quantity of a pharmaceutically-acceptable salt of a base according to formula I formed with a non-toxic acid is mixed with starch or other excipient and the mixture placed in telescoping gelatin capsules each containing from 7.5—50 mg. of active ingredients. Similarly, a pharmaceutically-acceptable salt can be mixed with starch, a binder, and a lubricant and the mixture compressed into tablets each containing from the 7.5—50 mgs. of salt. The tablets may be scored if lower or divided dosages are to be used. With parenteral administration, the intravenous route is preferred although with smaller mammals such as mice the intraperitoneal route is used. For parenteral administration, isotonic solutions are employed containing 1—10 mg./ml. of a salt of a compound of formula I. The compounds are administered at a rate of from 0.01 to 1 mg./kg. and preferably from 0.1 to 1 mg./kg. of mammalian body weight once or twice a week or every two weeks depending on both the activity and the toxicity of the drug. An alternative method of arriving at a therapeutic dose is based on body-surface area with a dose in the range 0.1 to 10 mg./meter squared of mammalian body surface every 7 or 14 days.

WHAT WE CLAIM IS:—

1. A dimeric indoledihydroindole of the formula

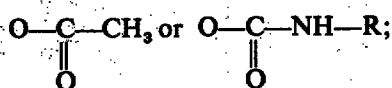


wherein

R is H, C₁—C₄ alkyl, C₃—C₄ alkenyl, CH₂—CHX—CH₃ or CH₂—CH₂X wherein

X is Br or Cl;

R¹ is OH,



R² is H, CH₃ or CHO;

either one of R³ and R⁴, when taken singly, is H or OH and the other C₂H₅ and

R⁵ is H;

or R⁴ and R⁵, taken together, form an epoxide ring and R³ is C₂H₅;

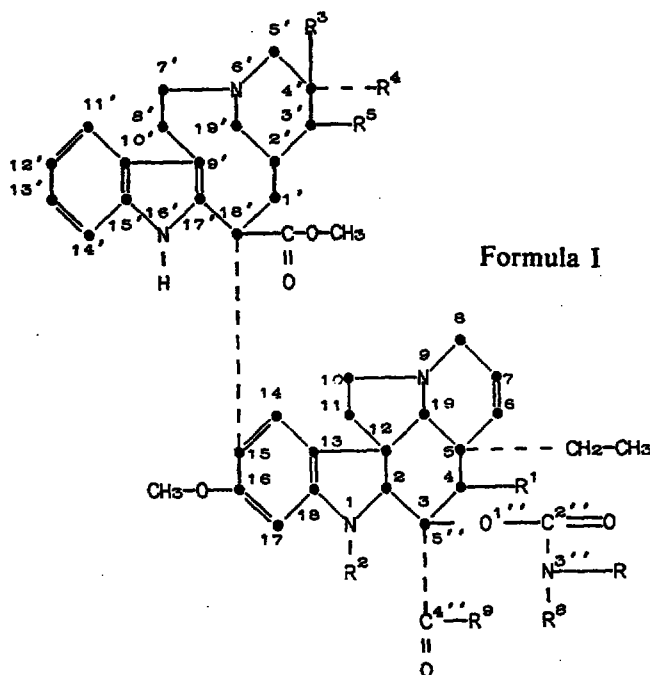


the intraperitoneal route) comparable tumor inhibition or prolongation of life is shown at the 0.5—0.75 mg./kg. dose levels for oxazolidine diones and at dose levels of 0.4—0.45 mg./kg for VLB and 0.2—0.25 for vincristine.

In utilizing the novel compounds of this invention as anti-tumor agents in mammals, either the parenteral or oral route of administration may be employed. For oral dosage, a suitable quantity of a pharmaceutically-acceptable salt of a base according to formula I formed with a non-toxic acid is mixed with starch or other excipient and the mixture placed in telescoping gelatin capsules each containing from 7.5—50 mg. of active ingredients. Similarly, a pharmaceutically-acceptable salt can be mixed with starch, a binder, and a lubricant and the mixture compressed into tablets each containing from the 7.5—50 mgs. of salt. The tablets may be scored if lower or divided dosages are to be used. With parenteral administration, the intravenous route is preferred although with smaller mammals such as mice the intraperitoneal route is used. For parenteral administration, isotonic solutions are employed containing 1—10 mg./ml. of a salt of a compound of formula I. The compounds are administered at a rate of from 0.01 to 1 mg./kg. and preferably from 0.1 to 1 mg./kg. of mammalian body weight once or twice a week or every two weeks depending on both the activity and the toxicity of the drug. An alternative method of arriving at a therapeutic dose is based on body-surface area with a dose in the range 0.1 to 10 mg./meter squared of mammalian body surface every 7 or 14 days.

WHAT WE CLAIM IS:—

1. A dimeric indoledihydroindole of the formula



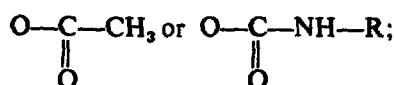
Formula I

25

wherein

R is H, C₁—C₄ alkyl, C₃—C₄ alkenyl, CH₂—CHX—CH₃ or CH₂—CH₂X wherein X is Br or Cl;

R¹ is OH,



30

R² is H, CH₃ or CHO;

either one of R³ and R⁴, when taken singly, is H or OH and the other C₂H₅ and R⁵ is H;

or R⁴ and R⁵, taken together, form an epoxide ring and R³ is C₂H₅;

25

30



23

15

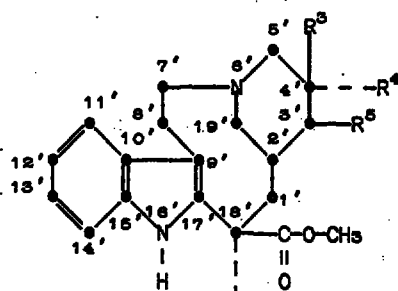
1,590,649

15

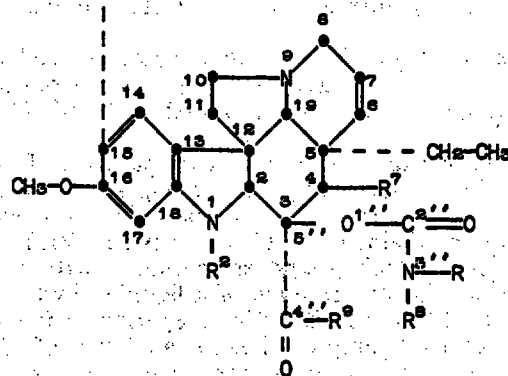
R^8 , taken singly, is H and R^8 , taken singly, is $-\text{O}-\text{CH}_3$; or R^8 and R^8 , taken together, are a single bond between $\text{C}^{4''}$ and $\text{N}^{3''}$; or pharmaceutically-acceptable salts thereof.

2. VLB 3''-methyl-3-spiro-5''-oxazolidine-2'',4''-dione.
3. VLB 3''-n-butyl-3-spiro-5''-oxazolidine-2'',4''-dione.
4. VLB 3''-allyl-3-spiro-5''-oxazolidine-2'',4''-dione.
5. Vincristine 3''-methyl-3-spiro-5''-oxazolidine-2'',4''-dione.
6. 1-desmethyl-4-desacetyl-4-(N-methylcarbamoyl) VLB 3''-methyl-3-spiro-5''-oxazolidine-2'',4''-dione.
7. VLB 3''-(β -chloroethyl)-3-spiro-5''-oxazolidine-2'',4''-dione.
8. VLB 3-N-(β -chloroethyl)-carbamate.
9. 4-desacetyl VLB 3''-methyl-3-spiro-5''-oxazolidine-2'',4''-dione.
10. 4-desacetyl-4-N-methylcarbamoyl-VLB 3''-methyl-3-spiro-5''-oxazolidine-2'',4''-dione.
11. 4-desacetyl VLB 3''- β -chloroethyl-3-spiro-5''-oxazolidine-2'',4''-dione.
12. VLB 3-spiro-5''-oxazolidine-2'',4''-dione.
13. The sulfate salt of a compound of formula I as defined in any of claims 1 to 12.

14. A process of preparing a dimeric indoleldihydroindole compound of the formula

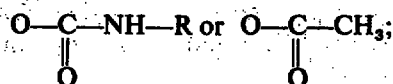


Formula III



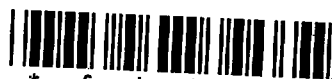
wherein

R , R^2 , R^3 , R^4 , R^5 , R^6 and R^8 are as defined in claim 1 and R^7 is



which comprises reacting a dimeric indoleldihydroindole of the formula

24

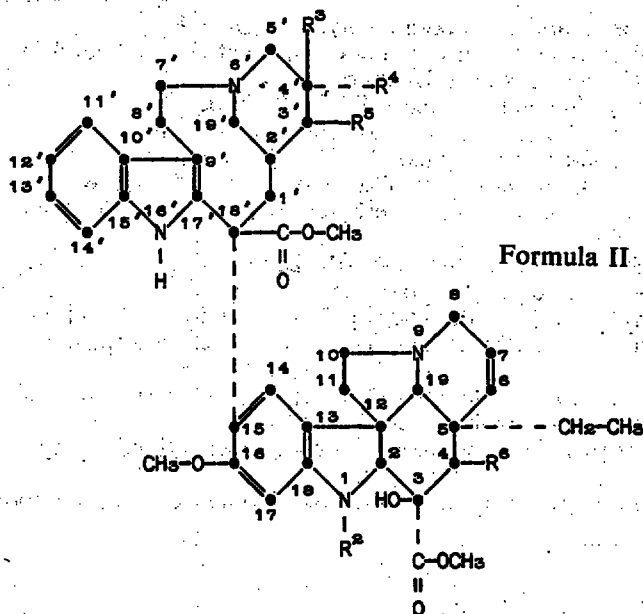


16

* C L M S *

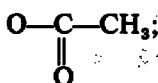
1,590,649

16



wherein

R^2 , R^3 , R^4 , and R^5 have the same meaning as hereinabove; and R^6 is OH or



with an isocyanate of the formula RNCO wherein R has the same meaning as above.

15. The process of Claim 14 wherein the dimeric indoledihydroindole dione and the isocyanate are mixed and heated to a temperature from 30 to 110°C. to form a spirooxazolidine dione of Formula III wherein R^6 and R^8 together are a bond between $C^{4''}$ and $N^{3''}$.

16. The process of Claim 14 or 15 for preparing VLB 3''-methyl-3-spiro-5''-oxazolidine-2'',4''-dione which comprises heating a mixture of VLB free base and methyl isocyanate in the presence of a liquid anhydrous reaction medium.

17. The process of Claim 14 or 15 for preparing VLB 3''-n-butyl-3-spiro-5''-oxazolidine-2'',4''-dione which comprises heating a mixture of VLB free base and n-butyl isocyanate in the presence of a liquid anhydrous reaction medium.

18. The process of Claim 14 or 15 for preparing VLB 3''-allyl-3-spiro-5''-oxazolidine-2'',4''-dione which comprises heating a mixture of VLB free base and allyl isocyanate in the presence of a liquid anhydrous reaction medium.

19. The process of Claim 14 or 15 for preparing vincristine 3''-methyl-3-spiro-5''-oxazolidine-2'',4''-dione which comprises heating a mixture of vincristine free base and methyl isocyanate in the presence of a liquid anhydrous reaction medium.

20. The process of Claim 14 or 15 for preparing 4-desacetyl-4-N-methyl-carbamoyl-VLB 3''-methyl-3-spiro-5''-oxazolidine-2'',4''-dione which comprises heating a mixture of 4-desacetyl VLB and methyl isocyanate in the presence of a liquid anhydrous reaction medium.

21. The process of any one of Claims 16 to 20 wherein the reaction medium is anhydrous benzene.

22. The process of Claim 14 or 15 for preparing 1-desmethyl-4-desacetyl-4-(N-methylcarbamoyl) VLB 3''-methyl-3-spiro-5''-oxazolidine-2'',4''-dione which comprises heating a mixture of 1-desmethyl-4-desacetyl VLB and methyl isocyanate in the presence of a liquid anhydrous reaction medium.

23. The process of Claim 22 wherein the reaction medium is anhydrous methylene dichloride.

24. The process of Claim 14 for preparing VLB 3''-(β-chloroethyl)-3-spiro-5''-oxazolidine-2'',4''-dione which comprises stirring a mixture of VLB free base and β-chloroethyl isocyanate in anhydrous benzene at room temperature, heating the



17

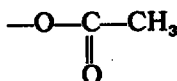
1,590,649

17

stirred mixture at reflux temperature, separating the complex of VLB and β -chloroethyl isocyanate thus formed and dissolving the complex in water.

25. The process of Claim 14 for preparing VLB 3''-(β -chloroethyl)-3-spiro-5''-oxazolidine-2'',4''-dione and VLB 3-N-(β -chloroethyl)-carbamate which comprises mixing VLB free base and β -chloroethyl isocyanate and heating the mixture at reflux temperature.

26. The process of any of Claims 14 to 25 which comprises the further step of subjecting a dimeric indole-dihydroindole of Formula I wherein R^1 is

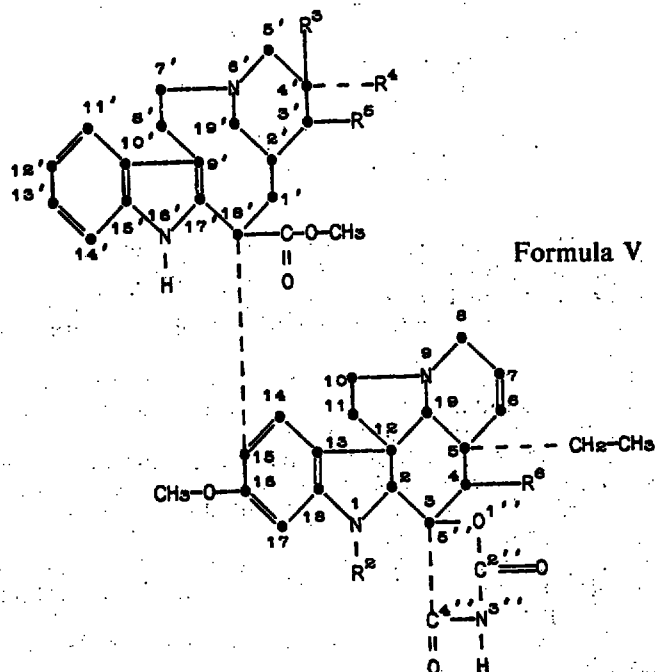


10

to acid hydrolysis to provide the corresponding 4-desacetyl derivative.

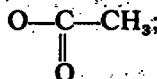
27. A process for preparing a spirooxazolidinedione of the formula

10



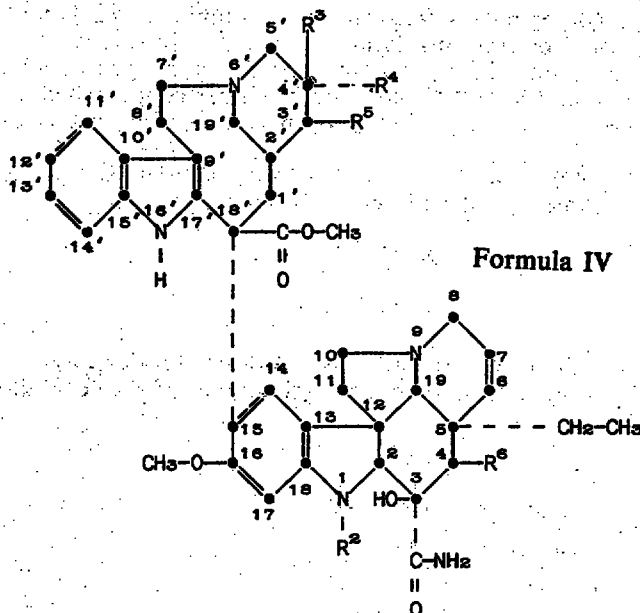
R^2 , R^3 and R^4 are as defined in claim 1 and R^5 is OH or

15



15

comprising reacting a dimeric indole-dihydroindole of the formula



wherein

R^2 , R^3 , R^4 , R^5 and R^6 have the same meaning as hereinabove; with dimethylcarbonate in the presence of sodium hydride.

28. The process of Claim 1 for preparing VLB 3-spiro-5''-oxazolidine-2'',4''-dione which comprises reacting vindesine with dimethyl carbonate in the presence of sodium hydride.

29. A pharmaceutical composition for the inhibition of tumors or prolongation of the life of the host mammal comprising a carrier and as active ingredient a compound of formula I according to Claim 1.

30. A method of inhibiting a tumor or prolonging the life of the host non-human mammal by the administration to a mammal host of a tumor of an anti-tumor effective dose of a dimeric indole dihydroindole compound of Formula I as defined in any one of Claims 1 to 14.

31. A dimeric indole-dihydroindole compound of the Formula I as defined in Claim 1 substantially as hereinbefore described with particular reference to any one of Examples 1 to 10.

32. A pharmaceutical composition as defined in Claim 29 substantially as hereinbefore described.

33. A process for preparing a dimeric indole dihydroindole compound of the Formula I as defined in Claim 14 substantially as hereinbefore described with particular reference to any of Examples 1 to 5 and 7.

34. A process for preparing a spirooxazolidine compound of the Formula V as defined in Claim 27 substantially as hereinbefore described with particular reference to Example 10.

35. A method of inhibiting a tumor or prolonging the life of the host non-human mammal as defined in Claim 30 substantially as hereinbefore described.

P. G. STRINGER,
Chartered Patent Agent,
Erl Wood Manor,
Windlesham,
Surrey, England.
Agent for the Applicants.

