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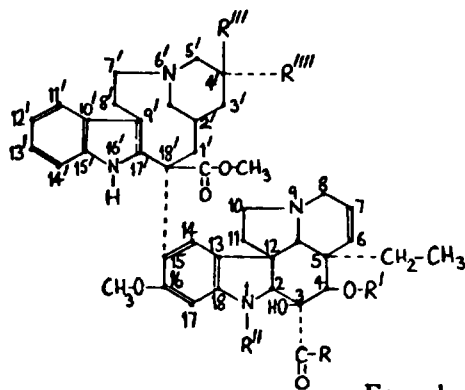


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(54) VINCA ALKALOID DERIVATIVES

(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:—
The invention relates to *Vinca* alkaloid derivatives and more particularly to derivatives of vinblastine, leurosidine and leurocristine, processes for the preparation thereof, pharmaceutical preparations containing the derivatives as the active ingredient and methods for treating neoplastic disease by administering the derivatives to a mammal.

The invention provides compounds of the following formula



Formula I

wherein R is NH₂, NH—NH₂, N(CH₃)₂, NH—alk—X, NH—(C₃—C₆)—cycloalkyl, NH—alk—Am, NH—alk—(OH)₁₋₃, or N₃; wherein alk is (C₂—C₆)—alkylene, Am is NH₂, NHCH₃, or N(CH₃)₂, X is hydrogen, cyano, optionally substituted phenyl carboxyl (COOH), carbo—(C₁—C₃)—alkoxy or carb-

oxamido (CO—NH₂), R' is H, (C₁—C₃)—alkyl—CO or chloro—(C₁—C₃)—alkyl—CO; wherein R'' is hydrogen, (C₁—C₃) alkyl, formyl or (C₁—C₃) alkyl—CO; and wherein one of R''' and R'''' is hydroxyl and the other is ethyl and salts thereof.

It is preferred for R to be NH₂, NH—NH₂, or NH—(C₁—C₃ alkyl); R' to be (C₁—C₃)—alkyl—CO or chloro—(C₁—C₃) alkyl—CO; R'' to be hydrogen, (C₁—C₃)alkyl or (C₁—C₃)—alkyl—CO; R''' to be OH and R'''' to be ethyl.

The invention further provides a process for preparing compounds of Formula I by reacting a compound of Formula I wherein R is O—CH₃, R' is hydrogen or acetyl and R'', R''', R'''' are as defined above with ammonia, methylamine or hydrazine, to obtain a compound of Formula I wherein R is NH₂, NH—CH₃, or NH—NH₂, and R' is H, and acylating, if desired, to form a compound of Formula I wherein R' is acetyl and, if desired, when R is NH—NH₂, (a) reacting said compound with a nitrosating agent to form an azide in which R is N₃, and then reacting said azide with ammonia, an amine of the formula HN(CH₃)₂, NH₂—alk—X, NH₂—(C₃—C₆)cycloalkyl, NH₂—alk—Am or NH₂—alk—(OH)₁₋₃, wherein alk is (C₁—C₆)—alkylene, Am and X are as defined above, to obtain the corresponding compound of Formula I, or (b) hydrogenolyzing said compound in which R is NH—NH₂ with Raney nickel in an inert solvent to yield a compound of Formula I wherein R is NH₂, R' is hydrogen or acetyl, and R'', R''' and R'''' are as defined above.

Further in accordance with the present invention there is provided a pharmaceutical formulation comprising a compound of Formula I as defined above or a pharmaceutically acceptable salt thereof in association with a pharmaceutically acceptable carrier therefor.

The invention also provides a pharmaceutical preparation in dosage unit form adapted for administration to obtain an anti-

neoplastic effect having per dosage unit, an effective amount within the range 0.1 to 1.0 mg/kg of mammalian body weight of a salt of the compound of Formula I in the presence of a pharmaceutical diluent.

The invention additionally provides a method of treating neoplasms by administering to a mammal suffering from a neoplastic disease an anti-neoplastically-effective amount of a compound of Formula I or salt thereof in the range of 0.1 to 1.0 mg/kg of mammalian body weight.

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 No. 3,370,057), vincalukoblastine (vinblastine) (U.S. Patent No. 3,097,137), leurosidine (vinrosidine) and leurocristine (vincristine) (both in U.S. Patent No. 3,205,220). Two of these alkaloids, vinblastine and leurocristine, are now marketed as drugs for the treatment of malignancies, particularly the leukemias and related diseases in humans. Of these marketed compounds, leurocristine is a most active and useful agent in the treatment of leukemias, but is also the least abundant of the anti-neoplastic alkaloids of *Vinca rosea*.

Chemical modification of the *Vinca* alkaloids has been rather limited. In the first place, the molecular structures involved are extremely complex and chemical reactions which affect a specific function of the molecule are difficult to develop. Secondly, alkaloids lacking desirable chemo-therapeutic properties have been recovered from *Vinca rosea* fractions, and a determination of their structures has led to the conclusion that these compounds are closely related to the active alkaloids. Thus, 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 correspondingly slight. Among the successful modifications of physiologically-active alkaloids has been the preparation of dihydro vinblastine (U.S. Patent No. 3,352,868) and the replacement of the acetyl group at C—4 (carbon no. 4 of the vinblastine ring system, see Formula I) with a higher alkanoyl group or with unrelated acyl groups. (See U.S. Patent No. 3,392,173.) Several of these derivations are capable of prolonging the life of mice inoculated with P1534 leukemia. One of the derivatives in which a chloroacetyl group replaced the C—4 acetyl group of vinblastine was also a useful intermediate for the preparation of structurally modified vinblastine compounds in which an N,N-dialkylglycyl group replaced the C—4 acetyl group of vinblastine (see U.S. Patent No. 3,387,001). An intermediate compound, namely 4-desacetyl vinblastine, was produced during the chemical reactions leading to these

latter derivatives. This intermediate, in which the C—4 acyl group was lacking, leaving an unesterified hydroxy group, has been reported to be a toxic material having little *in vivo* chemotherapeutic activity against the P1534 murine leukemia system by Hargrove, *Lloydia*, 27, 340 (1964).

Illustrative of $alk-(OH)_{1-3}$, $alk-Am$ and $alk-X$ in the above formula are the following: methyl, 2-methylpentyl, isohexyl, isopentyl, n-pentyl, n-hexyl, *sec*-hexyl, ethyl, isopropyl, n-butyl, *sec*-butyl, cyanomethyl, cyanoethyl, 2-hydroxy-n-hexyl, 5-cyano-n-pentyl, 2-hydroxyethyl, 3-hydroxypropyl, 2-dimethylaminoethyl, 2-aminoethyl, 2-methylaminoethyl, 2-hydroxypropyl, benzyl, phenethyl, 4-phenylbutyl, dimethylaminomethyl, 2-aminopropyl, 2-aminoethyl, 2-dimethylaminopropyl, 2,2-dihydroxyisopropyl, 2,2-dihydroxy-t-butyl, and 2,2,2-trihydroxy-t-butyl.

In the above formula, the terms " (C_1-C_3) -alkyl-CO" and "chloro- (C_1-C_3) -alkyl-CO" include groups such as acetyl, chloroacetyl, propionyl, 2-chloropropionyl, 2-chlorobutyryl and butyryl, these terms " (C_1-C_3) -alkyl-CO" representing an alkanoyl group and "chloro- (C_1-C_3) -alkyl-CO" representing a chloro-alkanoyl group. The term " $NH-(C_3-C_6)$ -cycloalkyl" includes the radicals cyclopropylamino, cyclobutylamino, cyclopentylamino, cyclohexylamino, cycloheptylamino and cyclooctylamino. The term "carbo- (C_1-C_3) -alkoxy" includes the radicals carbomethoxy, carboethoxy, carboisopropoxy and carbo-n-propoxy.

When "X" in the radical " $alk-X$ " is phenyl, the phenyl group can contain the standard aromatic substituents including C_1-C_4 alkyl, C_1-C_4 alkoxy, hydroxy, halo and nitro and a given phenyl group can contain more than one substituent, in which case the substituents may be either the same or different from one another; examples of such groups are 4-hydroxyphenyl, 2,4-dichlorophenyl, 2-methyl-4-chlorophenyl, 2,4-dinitrophenyl, 3,5-xylyl, 4-tolyl, 2-tolyl, and 3-ethoxyphenyl.

Pharmaceutically-acceptable acid addition salts of the amine bases may be prepared by reacting the compounds of Formula I with the acid. Such acids include inorganic acids such as: hydrochloric acid, nitric acid, orthophosphoric acid, sulfuric acid, hydrobromic acid, hydroiodic acid, nitrous acid, phosphorous acid, as well as non-toxic organic acids including aliphatic mono and dicarboxylic acids, phenyl-substituted alkanic acids, hydroxy alkanic acids and alkandioic acids, aromatic carboxylic acids, aliphatic and aromatic sulfonic acids. Such pharmaceutically-acceptable salts thus include the sulfate, pyrosulfate, bisulfate, sulfite, bisulfite, nitrate, orthophosphate, monohydrogenphosphate, dihydrogenphosphate, metaphosphate, pyrophosphate, chloride, bromide, iodide, acetate,

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 —Am and following: 75
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 isopropyl, 75
 cyanoethyl, 75
 entyl, 2- 75
 2-dimethyl- 80
 aminoethyl, 80
 , 4-phenyl- 80
 ninopropyl, 80
 opyl, 2,2- 80
 -butyl, and 85
 (C₁—C₃)- 85
 -alkyl-CO" 85
 chloroacetyl, 85
 chlorobutyryl 85
 -alkyl-CO" 90
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 a chloro- 90
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 cyclopropyl- 95
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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, (all isomers), methylbenzoate (all isomers), dinitrobenzoate (all isomers), hydroxybenzoate (all isomers), methoxybenzoate (all isomers), phthalate, terephthalate, benzenesulfonate, toluenesulfonate (all isomers), chlorobenzenesulfonate (all isomers), xylenesulfonate (all isomers), phenylacetate, phenylpropionate (all isomers), phenylbutyrate (all isomers), citrate, lactate, 2-hydroxybutyrate, glycolate, malate, tartrate, methanesulfonate, propanesulfonate, naphthalene-1-sulfonate and naphthalene-2-sulfonate.
 Compounds of the above formula can be described generally as either derivatives of vinblastine where R' is acetyl, R'' is methyl, R''' is hydroxyl and R'''' is ethyl, or of desacetyl vinblastine where R'', R''' and R'''' remain the same but R' is hydrogen or of leurocristine where R' is acetyl, R'' is formyl, R''' is hydroxyl and R'''' is ethyl, or of desacetyl leurocristine where R'', R''' and R'''' remain the same but R' is hydrogen, or as derivatives of desmethyl vinblastine (also known as desformylleurocristine) where R' is acetyl, R'' is hydrogen, and R''' and R'''' are hydroxyl and ethyl, respectively or of desacetyl desmethyl vinblastine (or desacetyl desformyl leurocristine) where both R' and R'' are hydrogen and R''' and R'''' are hydroxyl and ethyl, respectively, or of leurosine where R' is acetyl, R'' is methyl, R''' is ethyl and R'''' is hydroxyl, or of desmethyl leurosine where R', R''' and R'''' remain the same but R'' is hydrogen, or of desacetyl desmethyl leurosine where R' and R'' are hydrogen, R''' is ethyl and R'''' is hydroxyl, or of desacetyl leurosine where R' is hydrogen, R'' is methyl and R''' and R'''' are ethyl and hydroxyl, respectively. In each instance, the term "desacetyl" refers to the lack of an acetyl group on the hydroxyl at C—4 of the complex indole-dihydro-indole ring system.
 The derivatives exemplified by Formula I are those in which the carbomethoxyl group at C—3 of certain known indole-hydroindole alkaloids is transformed to a carboxhydrazide group, a carboxazide group, a carboxamide group or a derivative thereof. Not all of these derivatives are ordinarily prepared by one process. The compounds in which R in Formula I above is NH₂, NH—NH₂ or NH—CH₃ are prepared as follows: Treatment of vinblastine, leurocristine, leurosine, desmethyl vinblastine, desmethyl leurosine, or their respective 4-desacetyl compounds with either ammonia, methylamine or hydrazine yields the correspondingly 4-desacetyl amide, N-methylamide or hydrazide. The product of this reaction with starting materials having

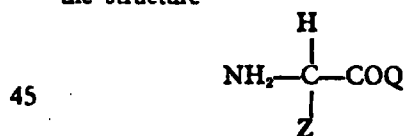
an intact 4-acetyl group is usually a compound in which the carbomethoxy group at C—3 is transformed to a carboxamide, N-methylcarboxamide or carboxyhydrazide group, but also in which the acetyl group at C—4 is completely removed.
 The compounds of this invention in which R is N(CH₃)₂, NH—alk—X wherein X is H, CN or phenyl, NH—(C₆—C₈)-cycloalk, NH—alk—Am or NH—alk—(OH)₁₋₃ and alk and Am are as previously defined are prepared by the following procedure: a hydrazide, of Formula I wherein R is NH—NH₂, prepared by reaction of the C—3 carbomethoxyl compound with anhydrous hydrazine, is transformed into the corresponding azide by treatment with a nitrosating agent such as nitrous acid, nitrosyl chloride, nitrogen tetroxide or amyl nitrite according to conventional procedures. The C—3 azide thus prepared is then reacted with the primary or secondary amines HN(CH₃)₂, NH₂—alk—X, NH₂—(C₆—C₈)-cycloalkyl, NH₂—alk—(OH)₁₋₃ or NH₂—alk—Am, to yield the desired C—3 amide coming within the scope of Formula I above. This C—3 azide-amine reaction does not affect the C—4 acyl group which if present remains intact during the reaction and work-up. The above azide-amine transformation follows the procedure originated by Stoll and Huffman, *Helv. Chim. Acta.*, 26, 944 (1943)—see also U.S. Patents 2,090,429 and 2,090,430.
 4-Desacetyl compounds of the invention can be prepared, as has been stated above, by reaction of vinblastine, leurocristine or leurosine directly with ammonia, methylamine or hydrazine, which will produce the 4-desacetyl derivative, and, in the case of the hydrazide, which is followed by conversion to the azide followed by reaction of the azide with an amine to yield the amides of Formula I. More generally, however, because of the lability of the 4-acetyl group under basic reaction conditions, the hydrazine-azide-amide reaction sequence is carried out with a 4-desacetyl derivative. In general, the 4-desacetyl amides according to Formula I above can be acylated with an aliphatic anhydride or acid chloride to yield the corresponding C—4 acetate, propionate or butyrate or a chloro derivative thereof. If R'' is hydrogen however, the acylated product will have R'' as (C₁—C₃)-alkyl-CO or chloro-(C₁—C₃)-alkyl-CO. An acid chloride (C₁—C₃)-alkyl-COCL or chloro-(C₁—C₃)-alkyl-CO-Cl or an acid anhydride [(C₁—C₃)-alkyl-CO]₂O or [chloro-(C₁—C₃)-alkyl-CO]₂O can be used in the acylation reaction. The preferred acylation procedure is that described in U.S. 3,392,173 for vinblastine or leurocristine in which a diacyl derivative is the first product of the reaction, and this derivative is selectively hydrolysed to yield a 4-acyl compound. Other procedures involving selective acylation

or multiple acylation followed by selective hydrolysis can be employed to prepare the 4-acyl derivatives.

- There are, however, certain provisos which must be kept in mind when an acylation procedure is contemplated. In the first place, as will be obvious to those skilled in the art, acylation of a 1-desformyl leurocristine (1-desmethyl vinblastine) or of a 1-desmethyl-leurosidine will result in acylation also at N—1. Therefore, a 1-desformyl or 1-desmethyl compound must be formylated, acylated or alkylated to give the desired substituent at N—1 prior to the C—4 acylation procedure. In addition if the C—3 carboxamide group contains an acylable group, i.e., hydroxy or amino, the C—4 acylation procedure must be carried out prior to the azide-amine reaction which yields the ultimate C—3 carboxamide group. The preferred procedure here is to acylate by the above procedures, the C—3 carboxhydrazide, first protecting the hydrazide group itself, which would otherwise also be acylated. The preferred hydrazide protecting group is the propylidene group formed by reaction of the NH₂ portion of the hydrazide moiety with acetone. This group can be readily removed by treatment with acid or, preferably, the propylidene derivative itself can be reacted directly with nitrite to form an azide group (see U.S. Patent 3,470,210, Example VII).

- Other procedures involving selective acylation or multiple acylation followed by selective hydrolysis or selective protection of an acylable function followed by acylation and subsequent removal of the protecting group will be apparent to those skilled in the art.

- Compounds according to Formula I above in which R is N—alk—X wherein alk is CH₂ or CH(C₁—C₃ alkyl), and X is carboxyl, carboxamido or carbo-(C₁—C₃)-alkoxy may advantageously be prepared by reacting an amino acid, amino ester or amino amide of the structure



- wherein Q is OH, NH₂ or O-alk and Z is H or a C₁—C₃ alkyl group, with the chosen dimeric indole-dihydroindole azide. Amino acids useful for this purpose, and coming within the scope of Formula I, include leucine, isoleucine, valine, glycine, alanine and nor-leucine. As will be apparent to those skilled in the art, other amino acids and polypeptides can also be used to react with, for example, 4-desacetyl vinblastine C—3 carboxazide, to yield substituted C—3 carboxamides having anti-tumor properties.

- An alternative method of preparing the primary amide (R is NH₂) from the hydrazide (R is NH—NH₂) involves the use of

a procedure based on that of Ainsworth, U.S. Patent 2,756,235, in which the hydrazide is hydrogenolyzed with Raney nickel.

The derivatives of Formula I will be named with reference only to the new group formed at a given carbon atom. For example, the compound produced by replacing the methyl ester function in vinblastine at C—3 with an amide function will be called simply vinblastine C—3 carboxamide, and not vinblastine C—3 descarbomethoxy C—3 carboxamide.

The compounds of Formula I, in the form of their free bases, including both carboxamides, carboxazides and carboxhydrazides are white or tan-coloured amorphous solids. It is preferable, however, where possible to isolate and crystallize the amides in the form of their anionic salts formed with non-toxic acids. Such salts are high-melting, white, crystalline or amorphous, water-soluble solids.

The preparation of the compounds of Formula I is more fully illustrated in the following specific examples:

Example 1.

Vinblastine C—3 N-methyl carboxamide and 4-desacetyl vinblastine C—3 N-methylcarboxamide.

A solution containing 21 g. of methylamine was prepared in 100 ml of anhydrous methanol at -78°C. About 3.5 g of vinblastine were added to this solution. The reaction vessel was sealed and placed in a constant temperature bath at 50°C for eight days. The reaction vessel was then opened, and the volatile contents removed by evaporation in vacuo. The nmr and infrared spectra of the residue indicated that it was a mixture of 4-desacetyl vinblastine C—3 N-methylcarboxamide and of 4-desacetyl vinblastine. The residue was dissolved in 100 ml. of pyridine, and 20 ml. of acetic anhydride were added. The resulting solution was allowed to stand at ambient temperature for about 18 hours. The volatile constituents were removed by evaporation in vacuo, and the resulting residue chromatographed over alumina using an ethyl acetate-chloroform (1:1) solvent mixture as the eluant. Fractions containing vinblastine C—3 N-methylcarboxamide as indicated by thin layer chromatography were combined, and the solvent evaporated therefrom in vacuo.

The above acetylation procedure not only re-acetylates the C—4 hydroxyl but also acetylates the C—3 hydroxyl of some of the 4-desacetyl vinblastine (C—3 N-methylcarboxamide in the above mixture. The acetyl group at C—3 was removed from the C—3 acetylated compound in the residue of the combined chromatographic fractions by the procedure of Hargrove, *Lloydia*, 27, 340 (1964), in which procedure the product containing C—3 acetylated material is treated with silica gel in aqueous methanol at room temperature for a period varying from 6 hours to several days

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5 to yield a product lacking the C—3 acetyl
 function. In this particular instance, the residue
 from the combined chromatographic fractions
 was dissolved in 50 ml of methanol. Twenty
 ml of water and 2 g. of silica gel were added.
 The resulting mixture was filtered, the filtrate
 evaporated to dryness in vacuo, and the
 resulting residue chromatographed over silica
 gel. A benzene-chloroform-triethylamine
 (100:50:7.5) solvent mixture was used as the
 eluant. Fractions containing the desired vin-
 blastine C—3 N-methylcarboxamide were
 collected, and the solvent removed therefrom
 by evaporation in vacuo. The residue was
 dissolved in aqueous methanol, and the pH
 adjusted to 2.9 with 1 percent sulfuric acid.
 Evaporation of the resulting mixture in vacuo
 yielded vinblastine C—3 N-methylcarbox-
 amide sulfate which crystallized from an-
 hydrous ethanol to yield material melting
 with decomposition at 272—5°C.
 An infrared spectrum of vinblastine C—3
 N-methylcarboxamide base obtained as indi-
 cated above showed a band at 1672 cm^{-1}
 (absent in the spectrum of vinblastine) indica-
 tive of the presence of an amide group. The
 nmr spectrum was in complete agreement
 with the proposed structure of vinblastine
 C—3 N-methylcarboxamide including the
 newly introduced N-methylcarboxamide func-
 tion which is represented by the sharp methyl
 resonance at 2.81 ppm.
 4-Desacetyl vinblastine C—3 N-methyl-
 carboxamide was isolated from the mixture
 with 4-desacetyl vinblastine obtained in the
 amidation step by the following procedure.
 The residue obtained by evaporation of the
 original (prior to reacylation) reaction mix-
 ture to dryness in vacuo was chromatographed
 over silica gel using as eluant the benzene-
 chloroform-triethylamine solvent system
 referred to above. Fractions shown by thin
 layer chromatography to contain 4-desacetyl
 vinblastine C—3 N-methylcarboxamide were
 combined and the solvent evaporated there-
 from in vacuo to yield the N-methyl carbox-
 amide as an amorphous solid. An infrared
 spectrum of the compound showed a band
 at 1572 cm^{-1} characteristic of an amide
 group. The absence of the 4-acetyl group was
 evidenced by the lack of resonance for this
 function at 2.1 ppm in the nmr (present in
 the nmr spectrum of vinblastine). The mole-
 cular weight determined by mass spectroscopy
 was 767 which is in agreement with the
 theoretical value calculated for $\text{C}_{44}\text{H}_{57}\text{N}_5\text{O}_7$.
 The sulfate salt of 4-desacetyl vinblastine
 C—3 N-methylcarboxamide was prepared by
 the procedure described for the preparation
 of vinblastine C—3 N-methylcarboxamide
 sulfate. The resulting product was an amor-
 phous, water-soluble solid.
 Following the above procedure, 2 g of
 4-desacetyl vinblastine were dissolved in a
 mixture of 75 ml of anhydrous methanol and

20 g. of ethyl amine. The reaction mixture
 was sealed and heated at about 60°C for
 about 8 days. 4-Desacetyl vinblastine C—3
 N-ethylcarboxamide thus obtained was sepa-
 rated by thin layer chromatography. The
 resulting solid showed a band at 1670 cm^{-1}
 in the infrared spectrum characteristic of the
 substituted carboxamide function.

Example 2.

Preparation of 4-desacetyl vinblastine C—3 carboxamide.

About 10 g of vinblastine sulfate were con-
 verted by standard procedures to vinblastine
 free base. The free base, obtained as a
 residue after evaporation of the dried ethereal
 solvent, was dissolved in about 200 ml of
 anhydrous methanol. Anhydrous liquid
 amounts (300 ml) was added, and the reaction
 mixture sealed and maintained at about 100°C
 for 60 hours. The reaction vessel was opened,
 and the contents removed and evaporated to
 dryness in vacuo. The resulting residue, was
 rechromatographed and fractions containing
 4-desacetyl vinblastine C—3 carboxamide, as
 shown by thin layer chromatography, were
 combined and the solvent evaporated there-
 from in vacuo, yielding as a residue purified
 4-desacetyl vinblastine C—3 carboxamide free
 base. The nmr and ir spectra of the solid
 free base confirmed the structure indicated. The
 free base showed a band in the infrared at
 1687 cm^{-1} , characteristic of the amide func-
 tion. The molecular weight of the free base
 determined by mass spectroscopy was 753
 which is in agreement with theoretical value
 calculated for $\text{C}_{43}\text{H}_{55}\text{N}_5\text{O}_7$.

600 mg of the above residue were converted
 to the sulfate salt in accordance with the
 procedure of Example 1. Evaporation of the
 reaction mixture to dryness yielded 4-desacetyl
 vinblastine C—3 carboxamide sulfate which
 crystallized from an ethanol-isopropyl solvent
 mixture and melted above 250°C. with de-
 composition. The salt was freely soluble in
 water.

4-Desacetyl vinblastine C—3 carboxamide
 was converted to vinblastine C—3 carbox-
 amide by the procedures of Example 1 as
 follows: 2.8 g of 4-desacetyl vinblastine C—3
 carboxamide free bases were acetylated with
 a mixture of anhydrous pyridine and acetic
 anhydride. The reaction mixture was main-
 tained for three days at room temperature.
 The volatile constituents were removed by
 evaporation in vacuo, and the resulting residue
 dissolved in methylene chloride. The methyl-
 ene chloride solution was washed with water,
 dried, and evaporated to dryness in vacuo,
 leaving as a residue vinblastine C—3 carbox-
 amide. The amide was purified by chroma-
 tography over silica gel using an ethyl acetate-
 ethanol (1:1) solvent mixture as the eluant.
 Fractions shown to contain the vinblastine
 C—3 carboxamide by thin layer chroma-

topography were combined, and the solvent removed therefrom by evaporation in vacuo, yielding a residue vinblastine C—3 carboxamide free base. The free base had the characteristic amide band in the infrared occurring at about 1700 cm^{-1} . Molecular weight by mass spectroscopy was 795 which is in agreement with the theoretical value calculated for $\text{C}_{33}\text{H}_{57}\text{N}_5\text{O}_8$. Vinblastine C—3 carboxamide sulfate was prepared by the method of Example 1 and crystallized from ethanol; m.p.=above 250°C .

Example 3.

4-Desacetyl vinblastine C—3 carboxhydrazide. Following the procedure of Example 1, 4-desacetyl vinblastine was heated in anhydrous ethanol with an excess of anhydrous hydrazine in a sealed reaction vessel at about 60°C for about 18 hours. The reaction vessel was cooled, and opened, the contents removed, and the volatile constituents evaporated therefrom in vacuo. The resulting residue, comprising 4-desacetyl vinblastine C—3 carboxhydrazine, was taken up in methylene chloride, the methylene chloride solution washed with water, separated and dried, and the methylene chloride removed by evaporation in vacuo. The resulting residue was dissolved in a 1:1 chloroform:benzene solvent mixture and chromatographed over silica gel. The benzene-chloroform-triethylamine eluant of Example 1 was employed to develop the chromatogram. The initial chromatographic fractions contained unreacted 4-desacetyl vinblastine. Further fractions were found to contain 4-desacetyl 18-descarbomethoxy vinblastine C—3 carboxhydrazide previously described by Neuss et al, *Tetrahedron Letters*, 1968, 783. The next fractions, found to contain 4-desacetyl vinblastine C—3 carboxhydrazide by thin layer chromatography, were combined, and the solvents evaporated therefrom in vacuo. The resulting solid melted at about $210\text{--}220^\circ\text{C}$. with decomposition. 4-Desacetyl vinblastine C—3 carboxhydrazide thus prepared had a carbomethoxy absorption band in the IR at $1725\text{--}1735\text{ cm}^{-1}$ thereby differentiating it from the 18-descarbomethoxy compound of Neuss et al *supra*, and a 1690 cm^{-1} band in the IR attributable to the hydrazide function. Molecular weight by mass spectroscopy was 768 in agreement with the theoretical value calculated for $\text{C}_{33}\text{H}_{56}\text{N}_6\text{O}_7$. The nmr spectrum contained the prominent resonance at 3.6 ppm representing the methyl group of the C—18 carbomethoxy function.

Following the above procedure, 4-desacetyl leurocristine available from Hargrove U.S. Patent 3,392,173 was reacted with anhydrous hydrazine in anhydrous methanol to yield 4-desacetyl 1-desformyl leurocristine C—3 carboxhydrazide, isolated as an amorphous powder. Infrared spectrum; absorption maxima at 1730 cm^{-1} (ester), 1670 cm^{-1} (hydrazide);

molecular ion spectrum: $m/e=754$ (consistent for $\text{C}_{33}\text{H}_{56}\text{N}_6\text{O}_7$); nmr $\delta 3.60$ (C_{18} methyl) $\delta 3.74$ (C_{16} methyl) $\delta 4.05$ (C_4 -hydrogen) $\delta 6.34$ (amide hydrogen).

Example 4.

4-Desacetyl leurocristine C—3 N-methylcarboxamide.

A solution of 900 mg of leurocristine and 473 ml of anhydrous methanol was saturated with gaseous hydrogen chloride at about 0°C . The reaction flask was then fitted with a drying tube and warmed to room temperature. After being maintained at that room temperature for about 24 hours, the volatile constituents were removed by evaporation in vacuo, and the resulting residue dissolved in water. The aqueous solution so formed was made basic with 14 N ammonium hydroxide and the alkali insoluble bases present extracted with methylene chloride. The methylene chloride extracts were combined, dried, and evaporated to dryness in vacuo. The resulting residue, comprising 4-desacetyl 1-desformyl leurocristine, was a light brown amorphous solid. Molecular weight by mass spectroscopy was 754 in agreement with that calculated for $\text{C}_{33}\text{H}_{54}\text{N}_4\text{O}_4$. In the infrared, an amide band at 1670 cm^{-1} , characteristic of the 1-formyl group, was lacking, but there was strong band at 1730 cm^{-1} , characteristic of ester absorption. Likewise the nmr spectrum showed peaks at 3.61 ppm and 3.85 ppm characteristic of the two carbomethoxy groups present; there was an additional peak at 4.11 ppm, characteristic of the proton at C—4.

A solution was prepared containing 500 mg of 4-desacetyl 1-desformyl leurocristine prepared as above in 75 ml of anhydrous methanol previously saturated with methylamine at -78°C . (The quantity of methylamine present was about 20 g.) The reaction vessel was sealed and heated at 60°C . for about one week. The volatile constituents were then removed by evaporation in vacuo to yield 4-desacetyl 1-desformyl leurocristine C—3 N-methylcarboxamide, a light brown amorphous solid. Infrared spectroscopy of the above compound showed a strong amide band at 1668 cm^{-1} in addition to an ester band at 1730 cm^{-1} .

4-Desacetyl 1-desformyl leurocristine C—3 N-methylcarboxamide thus prepared was formulated with a mixture of 24 ml of 97% formic acid and 2 ml of acetic anhydride. The reaction mixture was allowed to stand at room temperature for about 24 hours, after which time the volatile constituents were removed in vacuo. The residue thus obtained was dissolved in water, and the aqueous solution made basic with 14 N ammonium hydroxide. The nitrogenous bases insoluble in the alkaline solution were extracted into methylene chloride. The methylene chloride extracts were combined,

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crystalline and
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about 0°C.
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temperature. 75
room tempera-
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tion in vacuo,
dried in water. 80
was made
dichloride and
it extracted
methylene
dried, and 85
the resulting
1-desformyl
amorphous
mass spectro-
metric calcu- 90
lating infrared, an
characteristic of
but there
characteristic
nmr spectrum 95
3.85 ppm
hydroxy groups
peak at 4.11
at C-4.
using 500 mg 100
crystalline pre-
dried meth-
nethylamine
methylamine
reaction vessel 105
for about
s were then
so to yield
the C-3 N-
amorphous 110
the above
de band at
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of 24 ml
2 ml of
in mixture
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which time
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made basic
The nitro- 125
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chloride. The
combined,

dried, and the methylene chloride removed therefrom by evaporation in vacuo to yield 4-desacetyl leurocristine C-3 N-methylcarboxamide, a light brown amorphous solid having the following characteristics:

5 Molecular weight by mass spectrography was 781 in agreement with the theoretical value calculated for 4-desacetyl leurocristine C-3 N-methylcarboxamide, C₁₄H₁₃N₃O₇.

10 The nmr spectrum had peaks at 3.65 ppm, characteristic of the carbomethoxy group, and at 2.79 ppm, characteristic of the N-methylcarboxamide group.

15 Infrared spectrum contained a strong amide band at 1678 cm⁻¹, indicating the presence of more than one amide function in the molecule, and a characteristic ester band at 1728 cm⁻¹.

20 Following the procedure of Example 1, 4-desacetyl leurocristine C-3 N-methylcarboxamide free base was converted to the corresponding sulfate salt with one percent sulfuric acid. The sulfate was a white amorphous powder.

25 In the above procedure, other amide derivatives of 4-desacetyl 1-desformyl leurocristine C-3 N-methylcarboxamide can be formed by substituting acetic or propionic anhydride for the formylation reagent specified above to form the corresponding 4-desacetyl 1-desformyl 30 1-acetyl (or 1-propionyl) leurocristine C-3 carboxamide derivative. Surprisingly, reacylation of a 4-desacetyl leurocristine does not affect the 1-formyl group which remains intact during the synthetic procedure,

Example 5.

35 4-Desacetyl vinblastine C-3 carboxazide.

A solution of 678 mg. of 4-desacetyl vinblastine C-3 carboxhydrazide (from Example 3) was prepared in 15 ml. of anhydrous methanol. About 50 ml. of 1N aqueous hydrochloric acid were added, and the resulting solution cooled to about 0°C. Approximately 140 mg. of sodium nitrite were then added, and the resulting reaction mixture stirred for 10 minutes while maintaining the temperature at about 0°C. The solution turned dark red-brown upon the addition of the sodium nitrite. The reaction mixture was next made basic by the addition of an excess of cold 5 percent aqueous sodium bicarbonate. The aqueous solution was extracted three times with methylene dichloride. 4-Desacetyl vinblastine C-3 carboxazide formed in the above reaction passed into the methylene dichloride.

55 While ordinarily the methylene dichloride solution of 4-desacetyl vinblastine C-3 carboxazide is used without further purification, an aliquot was treated as follows in order to characterize the azide: Evaporation of the methylene dichloride left the azide in an amorphous state. The azide residue was washed with ether, and the resulting suspension filtered. The residual tan powder had the following distinguishing physical characteris-

tics: ultraviolet spectrum $\lambda_{max}=260$ mu. (epsilon=16,700); shoulder at about 290 mu; 309mu. (epsilon=7,100); infrared absorption maximum at about 1690 cm⁻¹ (carboxhydrazide) was absent, while the maximum at 1730 cm⁻¹ was not affected. Furthermore, a sharply defined maximum at 2135 cm⁻¹ was noted characteristic of the carboxazide function. The mass spectrogram revealed a molecular ion m/e=708 showing a loss of 71 mass units (H, CON₂) from the molecular weight calculated for C₁₄H₁₃N₃O₇=779. 4-Desacetyl vinblastine C-3 carboxazide sulphate in also within the scope of the invention.

Example 6.

4-Desacetyl vinblastine C-3 N-ethylcarboxamide.

A solution of 4-desacetyl vinblastine C-3 carboxazide was prepared in methylene dichloride solution according to the above procedure from 900 mg. of 4-desacetyl vinblastine C-3 carboxhydrazide. The methylene dichloride solution was dried, and the volume reduced to about 20 ml.

The solution of the azide in methylene dichloride was then placed in a flask fitted with a drying tube and stirrer. 50 ml. of anhydrous ethylamine were added thereto, and the reaction mixture was stirred at room temperature for about two hours. Evaporation of the volatile constituents *in vacuo* yielded a tan amorphous powder which was chromatographed over silica gel. The chromatogram was developed with an ethyl acetate-anhydrous ethanol (3:1) solvent mixture. Fractions containing 4-desacetyl vinblastine C-3 N-ethylcarboxamide as determined by thin-layer chromatography were combined, and the solvent was removed from the combined fractions *in vacuo*. 450 mg. of a tan amorphous powder was obtained with the following distinctive physical characteristics: molecular ion spectrum, m/e=781 (corresponding to C₁₆H₁₉N₃O₇); infrared spectrum; absorption maxima at 1730 cm⁻¹ (ester), 1670 (cm⁻¹ (amide), 3420 cm⁻¹ (N-H amide), nmr. 81.18 (triplet-β-methyl of ethyl amide group), 83.28 (quartet-α-methylene of ethyl amide group), 83.59 (singlet-methyl ester).

4-desacetyl vinblastine C-3 N-ethylcarboxamide sulfate was prepared by dissolving the above amorphous powder in anhydrous ethanol and adjusting the pH to about 4.0 with 2 percent sulfuric acid in anhydrous ethanol. Evaporation of the solvent *in vacuo* yielded a water-soluble tan powder comprising 4-desacetyl vinblastine C-3 N-ethylcarboxamide sulfate.

Following the above procedure, 4-desacetyl vinblastine C-3 N-isopropylcarboxamide was prepared. The compound had the following distinguishing characteristics: molecular ion spectrum, m/e=795 (corresponding to C₁₆H₂₁N₃O₇); infrared spectrum: maxima at

1730 cm^{-1} (ester); 1660 cm^{-1} (amide); nmr; δ 1.16, δ 1.22 (doublet for isopropyl methyl groups); 4-desacetyl vinblastine C—3 N-isopropylcarboxamide sulfate was prepared by the above procedure and was a tan water-soluble amorphous powder.

Following the above procedure, 4-desacetyl vinblastine C—3 N,N-dimethylcarboxamide was prepared having the following distinctive physical characteristics: molecular ion spectrum; $m/e=781$ (consistent with $\text{C}_{33}\text{H}_{49}\text{N}_5\text{O}_7$; infrared spectrum: absorption maxima at 1730 cm^{-1} (ester), 1620 cm^{-1} (amide) nmr δ 2.96 (singlet-N-methyl) δ 3.46 (singlet-N-methyl). The sulfate salt of 4-desacetyl vinblastine C—3 N,N-dimethylcarboxamide was prepared by the above procedure and was a water-soluble, tan amorphous powder.

Following the above procedure, 4-desacetyl vinblastine C—3 N—[2-(N,N-dimethylamino)ethyl]carboxamide was prepared from the carboxazide and N,N-dimethyl ethylene diamine, it had the following distinguishing physical characteristics: Infrared spectrum; absorption maxima at 3410 cm^{-1} (N—H amide), 1740 cm^{-1} (ester), 1670 cm^{-1} (amide); nmr δ 2.44, δ 3.41 (methylenes from ethylidene function) δ 2.23 (methyls of dimethylamino group); molecular ion spectrum; $m/e=824$ (consistent with $\text{C}_{31}\text{H}_{44}\text{N}_6\text{O}_7$); $\text{pK}_a=4.85, 7.0, 8.5$. The corresponding sulfate salt prepared by the above procedure was a tan, water-soluble amorphous powder.

4-Desacetyl vinblastine C—3 N-benzylcarboxamide was prepared using benzylamine by the above procedure and had the following distinctive physical characteristics: Infrared spectrum; maxima at 3420 cm^{-1} (N—H amide), 1735 cm^{-1} (ester), 1675 cm^{-1} (amide); nmr; δ 7.32 (aromatic protons) δ 3.69 (methylene group of benzyl amide); molecular ion spectrum, $m/e=843$ consistent with $\text{C}_{30}\text{H}_{41}\text{N}_5\text{O}_7$. The corresponding sulfate salt was made by the above procedure and was a water-soluble, tan amorphous powder.

Following the above procedure, 4-desacetyl vinblastine C—3 N-cyanomethylcarboxamide was prepared by reacting 4-desacetyl vinblastine C—4 carboxazide with cyanomethylamine. The novel amide had the following distinctive physical characteristics: Infrared absorption maxima at 1690 cm^{-1} (amide); 3420 cm^{-1} (amide NH); nmr; δ 4.17 ($\text{C}_4\text{—H}$); δ 2.80 (N—CH_3) δ 3.77 (aromatic H) δ 3.69 (methyl ester H) δ 4.48 ($J=6\text{Hz}$) δ 3.92 ($J=17\text{Hz}$); both cyanomethylene. Molecular ion spectrum $m/e=792$ (consistent with $\text{C}_{31}\text{H}_{40}\text{N}_5\text{O}_7$).

4-Desacetyl vinblastine C—3 N-(2-hydroxyethyl) carboxamide can also be prepared, and is within the scope of the invention.

Following the procedure of Example 5, 1-desformyl 4-desacetyl leurocristine C—3 carboxhydrazide, furnished by the procedure of Example 3, was reacted with sodium nitrite

in dilute hydrochloric acid to form 1-desformyl 4-desacetyl leurocristine C—3 carboxazide which was obtained as a yellow amorphous powder. A methylene dichloride solution of 1-desformyl 4-desacetyl leurocristine C—3 carboxazide was then reacted with ethylamine to yield 1-desformyl 4-desacetyl leurocristine C—3 N-ethylcarboxamide. The compound was purified by chromatography over silica gel using an ethyl acetate-anhydrous ethanol (1:1) solvent mixture as the eluant. Fractions, determined by thin-layer chromatography to contain the N-ethyl amide, were combined, and the combined fractions evaporated to dryness. 1-Desformyl 4-desacetyl leurocristine C—3 N-ethylcarboxamide was obtained as a yellow amorphous powder having the following distinctive physical characteristics: Infrared spectrum; absorption maxima at 1665 cm^{-1} (amide), 1745 cm^{-1} and 1730 cm^{-1} (ester bands, 1 hydrogen bonded), 3430 cm^{-1} (N—H amide); molecular ion spectrum; $m/e=767$ (consistent with $\text{C}_{34}\text{H}_{51}\text{N}_5\text{O}_7$).

Example 7.

4-Desacetyl leurosidine C—3 carboxhydrazide.

Following the method of Example 3, 1500 mg of leurosidine were reacted with 25 ml. of anhydrous hydrazine in anhydrous methanol solution. The reaction mixture was sealed in a reaction flask, and the flask heated to 50°C. for 12 days. Evaporation of the volatile constituent *in vacuo* yielded 4-desacetyl leurosidine C—3 carboxhydrazide having the following distinctive physical characteristics: Infrared maxima 1735 cm^{-1} (ester), 1660 cm^{-1} (hydrazide); molecular ion spectrum; $m/e=768$ (consistent with $\text{C}_{33}\text{H}_{48}\text{N}_6\text{O}_7$).

4-Desacetyl leurosidine C—3 carboxhydrazide was converted to the corresponding azide by treatment with sodium nitrite, and the azide reacted at -78°C with methanol saturated with ammonia to yield 4-desacetyl leurosidine C—3 amide which was purified by chromatography as before. The compound had the following physical characteristics: Infrared maxima 3400 cm^{-1} (N—H amide) 1740 cm^{-1} (ester), 1690 cm^{-1} (amide); molecular ion spectrum, $m/e=753$ (consistent with $\text{C}_{33}\text{H}_{48}\text{N}_5\text{O}_7$); δ 3.58 (C_{11} , methyl ester) δ 3.78 (C_{16} , methoxyl), δ 2.88 (C_1 , methyl), δ 5.78 (C—3 amide hydrogens), δ 4.18 (C_4 hydrogen). The corresponding sulfate salt was prepared by a procedure involving neutralization of an ethanol solution of the base with ethanolic sulfuric acid to yield a tan amorphous powder.

Other amides of leurosidine can be prepared in similar fashion.

Each of the desacetyl 1-desformyl 1-alkanol derivatives, as well as the 4-desacetyl vinblastine, leurocristine, or leurosidine C—3 carboxamides with exception of the carboxhydrazides and those amide moieties containing a reactive function as discussed above, can

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in e line
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—H amide)
—1 (amide);
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64.18 (C,
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be reacted with other anhydrides such as chloroacetic acid anhydride, butyric anhydride and 2-chloropropionic anhydride to yield using, for example, chloroacetic anhydride, a mixture of the 3,4-bis-chloroacetyl and the 4-chloroacetyl derivatives which can be converted to the pure 4-chloroacetyl derivative by treatment with wet silica gel. Other 4-acyl derivatives are prepared in similar fashion.

Example 8.

Preparation of salts.

Other salts, including salts with inorganic anions such as chloride, bromide, orthophosphate and nitrate, as well as salts with organic anions such as acetate, chloroacetate, trichloroacetate, benzoate, and alkyl or aryl sulfonates are prepared from the amide bases by a procedure analogous to that set forth in Example 1 above for the preparation of the sulfate salt by substituting the appropriate acid in a suitable diluent in place of the 1 percent aqueous sulfuric acid of that example.

As will be apparent to those skilled in the art, the presence of other ester and/or amide groups in the indole-dihydroindole components requires extra care in the preparation of salts so as to avoid hydrolysis, transesterification and other reactions which take place at high temperatures, extremely acid pH's etc.

The compounds have shown antiviral activity in vitro against herpes virus employing a tissue culture system in a plaque suppression test similar to that described by Siminoff, *Applied Microbiology*, 9, 66—72 (1961). For example, vinblastine C—3 carboxamide sulfate gave a 20 mm inhibition zone with a 3 rating and without toxicity at a dose level of 125 $\mu\text{g}/\text{ml}$. The same compound was shown to be able to induce metaphase arrest in cultured Chinese hamster ovary cells at doses ranging from 2×10^{-2} $\mu\text{g}/\text{ml}$ to 2×10^{-3} $\mu\text{g}/\text{ml}$. A most active inducer of such arrest was shown to be 4-deacetyl leurocristine C—3 N-methyl-

carboxamide sulfate which was effective at doses on the order of 10^{-6} $\mu\text{g}/\text{ml}$.

In addition, the compounds of this invention have been shown to be active against transplanted mouse tumors in vivo. For example, 4-desacetyl vinblastine C—3 carboxamide sulfate, 4-desacetyl vinblastine C—3 N-methylcarboxamide sulfate, vinblastine C—3 carboxamide sulfate, vinblastine C—3 N-methylcarboxamide sulfate, 4-desacetyl vinblastine C—3 N-(2-hydroxyethyl) carboxamide sulfate, 4-desacetyl vinblastine C—3 N,N-dimethylcarboxamide sulfate, 4-desacetyl leurosidine C—3 amide sulfate, 4-desacetyl vinblastine C—3 N-benzyl-carboxamide sulfate and 4-desacetyl vinblastine C—3 carbox-hydrazide, as well as other compounds coming within the scope of the above formula, demonstrated such activity. Of particular interest, however, is the activity of the compounds of this invention, especially the 4-desacetyl vinblastine C—3 carboxamide sulfate, and its N-alkyl and N-hydroxyalkyl derivative against Ridgeway osteogenic sarcoma (ROS) and Gardner lymphosarcoma (GLS). In demonstrating activity of the drugs of this invention against these tumors, 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 inoculation 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 Formula I. 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; and column 4, the percent inhibition of tumor growth. (ROS is an abbreviation for Ridgeway osteogenic Sarcoma; GLS for Gardner lymphsarcoma; and CA 755 is an adenocarcinoma).

TABLE I

Compound	Tumor	Dose mg/kg. $\times$ days	Percent Inhibition
4-Desacetyl vinblastine C-3 N-methylcarboxamide sulfate	C ₃ H mammary ROS GLS	0.25-0.3 $\times$ 9 0.15-0.3 $\times$ 10 0.05-0.4 $\times$ 8	35-49 48-100 60-100
Vinblastine C-3 carboxamide sulfate	ROS GLS	0.1-0.5 $\times$ 10 0.05-0.8 $\times$ 8	23-84 47-100
4-Desacetyl leurocristine C-3 N-methylcarboxamide sulfate	C ₃ H mammary CA755 GLS ROS	1.0 $\times$ 9 1.0 $\times$ 9 0.3-1.0 $\times$ 8 0.3-1.0 $\times$ 10	36 76 57-100 39-100
4-Desacetyl vinblastine C-3 carboxamide sulfate	GLS ROS	0.15-1.0 $\times$ 10 0.15-0.4 $\times$ 10	79-100 49-100
4-Desacetyl vinblastine C-3 N-ethylcarboxamide sulfate	GLS ROS	0.2 $\times$ 7 0.2-0.4 $\times$ 7	90 53-100
4-Desacetyl vinblastine C-3 N-benzylcarboxamide sulfate	ROS	0.3 $\times$ 10	100
Vinblastine C-3 N-methylcarboxamide sulfate	GLS ROS	0.1-0.25 $\times$ 8 0.1-0.3 $\times$ 10	38-100 57-100
4-Desacetyl vinyblastine C-3 carboxhydrazide	ROS GLS	0.1-0.3 $\times$ 8-10 0.2-0.4 $\times$ 10	49-100 73-100
4-Desacetyl vinblastine C-3 N-[2-hydroxyethyl]carboxamide sulfate	ROS	0.05-0.4 $\times$ 10	100

TABLE 1 (Continued)

Compound	Tumor	Dose mg/kg. $\times$ days	Percent Inhibition
4-Desacetyl vinblastine C-3 N,N-dimethylcarboxamide sulfate	ROS	$0.4 \times .0$	53
4-Desacetyl leurosine C-3 carboxamide sulfate	ROS	5×10	32
4-Desacetyl vinblastine C-3 cyanomethylcarboxamide sulfate	ROS	0.3×10	85

The compounds of Formula I, like leurocristine and vinblastine, are toxic to mice at doses above those at which they produce 100 percent inhibition of the transplanted tumor. In addition, for reasons that are not well understood, all drugs in a given test including control drugs like vinblastine may show toxicity at dose levels where they ordinarily give tumor inhibition without toxicity. Thus, the results set forth in Table 1 are of typical experiments where the control drugs give expected results and are not an average of all runs.

The compounds of Formula I are also active against other transplanted tumors. For example, with Mecca lymphosarcoma, parental injection of 0.25 mg/kg for 9 days of vinblastine C-3 N-methylcarboxamide sulfate gave a 54 percent inhibition of growth and of vinblastine C-3 amide, a 28 percent inhibition. At the same dose levels, vinblastine itself was completely inactive.

In addition, in studies against CA 755 adenocarcinoma, 4-desacetyl vinblastine C-3 carboxamide sulfate gave a 67 percent inhibition of tumor growth, 4-desacetyl vinblastine

C-3 N-methylcarboxamide sulfate 61 percent inhibition and vinblastine C-3 carboxamide sulfate 49 percent inhibition at a dose level of 0.25 mg/kg for eight days, and 72 percent inhibition at 0.3 mg/kg. In a similar experiment, vinblastine gave a 31 percent inhibition while leurocristine at the somewhat lower dose level of 0.2 mg/kg gave 79 percent inhibition with an excellent effectiveness rating. Against L5178Y lymphocytic leukemia, vinblastine C-3 carboxamide sulfate at a dose level of 0.25 mg/kg for ten days in an experiment using five mice gave three indefinite survivors; the life span of the two diseased mice in this experiment was prolonged by 26 percent over that of the control mice. In the same experiment, vinblastine gave a 36 percent prolongation but with no indefinite survivors and rated only a minimal effectiveness rating.

As would be expected, the amides and hydrazides differ in their anti-tumor spectrum from vinblastine leurocristine and leurosine, as well as from the C-4, N,N-dialkyl-glycyl esters of vinblastine in the same way that the anti-tumor spectra of those compounds differ among themselves, some being more effective

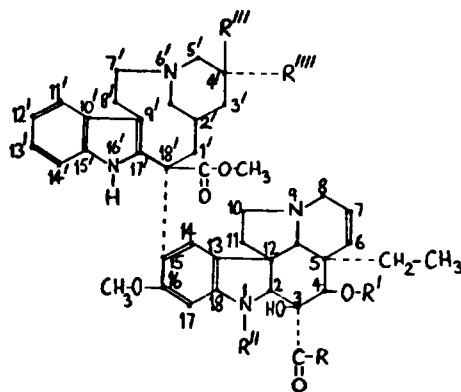
against certain tumors or classes of tumors and less effective against others.

- In utilizing the amides and hydrazides as anti-neoplastic agents, either the parenteral or oral route of administration may be employed.
- 5 For oral dosage, a suitable quantity of a pharmaceutically-acceptable salt of a base according to Formula I except those in which R is NH—NH₂ or N₃, formed with a non-toxic acid is mixed with starch or other
- 10 excipient and the mixture placed in telescoping gelatin capsules each containing from 7.5—50 mg of active ingredients. Similarly, the anti-neoplastic salt can be mixed with starch,
- 15 a binder, and a lubricant and the mixture compressed into tablets each containing from 7.5—50 mgs. The tablets may be scored if lower or divided dosages are to be used. With parenteral administration, the intravenous
- 20 route is preferred. For this purpose, isotonic solutions are employed containing 1—10 mg/ml of a salt of an indole-dihydroindole amide of Formula I except for the hydrazides and azides. The compounds are administered
- 25 at the rate of from 0.1 to 1 mg/kg mammalian body weight once a week, depending on both the activity and the toxicity of the drug. Free bases of compounds according to Formula I in which R is NH—NH₂ or N₃ are com-
- 30 pounded into suitable dosage forms and administered in similar fashion at similar dose levels.

While most of the compounds are useful as anti-neoplastic or antiviral drugs, two types of derivatives, the hydrazides and azides (compounds of Formula I wherein R is NH—NH₂ or N₃), are also useful as intermediates as has been set forth above, in that the hydrazide can be transformed to the

40 azide by reaction with a nitrosating agent, or to the simple amide by hydrogenolysis. The azide can in turn be made to react with primary or secondary amines to yield the amides of this invention.

45 **WHAT WE CLAIM IS:—**
1. A compound of the formula



Formula I

wherein R is NH₂, NH—NH₂, N(CH₃)₂, NH—alk—X, NH(C₃—C₆)cycloalkyl, NH—alk—Am, NH—alk—(OH)₁₋₃, or N₃; wherein alk is (C₁—C₆) alkylene, Am is NH₂, NHCH₃ or N(CH₃)₂, and X is hydrogen, cyano, optionally substituted phenyl, carboxyl, carbo (C₁—C₃) alkoxy or carboxamide; wherein R' is H, (C₁—C₃)—alkyl—CO or chloro—(C₁—C₃)—alkyl—CO; wherein R'' is hydrogen, C₁—C₃ alkyl, formyl or (C₁—C₃)—alkyl—CO; and wherein one of R''' and R'''' is hydroxyl and the other is ethyl; or a salt thereof.

2. A compound as claimed in claim 1, wherein R is NH₂, NH—NH₂ or NH—(C₁—C₃ alkyl); R' is (C₁—C₃) alkyl—CO or chloro (C₁—C₃) alkyl—CO; R'' is hydrogen, (C₁—C₃) alkyl or (C₁—C₃) alkyl—CO; R''' is OH and R'''' is ethyl.

3. A pharmaceutically-acceptable salt of a compound according to claim 1 or 2.

4. A sulfate salt of a compound according to claim 1, 2 or 3.

5. 4-Desacetyl vinblastine C—3 carboxamide.

6. 4-Desacetyl vinblastine C—3 carboxamide sulphate.

7. Vinblastine C—3 N—methylcarboxamide.

8. Vinblastine C—3 N—methylcarboxamide sulphate.

9. 4-Desacetyl leurocristine C—3 N-methylcarboxamide.

10. 4-Desacetyl leurocristine C—3 N-methyl carboxamide sulphate.

11. 4-Desacetyl vinblastine C—3 carboxhydrazide.

12. 4-Desacetyl vinblastine C—3 carboxazide.

13. 4-Desacetyl vinblastine C—3 carboxazide sulphate.

14. 4-Desacetyl vinblastine C—3 N-(2-hydroxyethyl) carboxamide.

15. A compound of Formula I, substantially as hereinbefore described with reference to any one of the foregoing Examples.

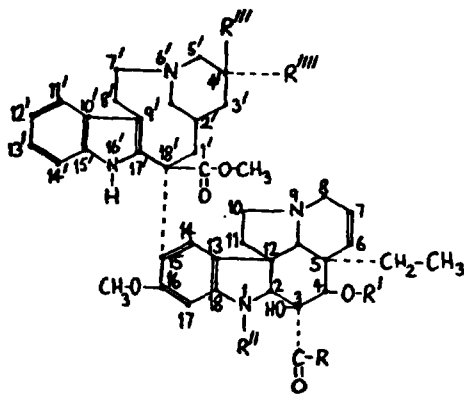
16. A process for preparing the compound of Formula I as defined in Claim 1 which comprises reacting a compound of Formula I wherein R is O—CH₃, R' is hydrogen or acetyl and R'', R''' and R'''' are as defined in Claim 1 with ammonia, methylamine or hydrazine to obtain a compound of Formula I wherein R is NH₂, NHCH₃ or NH—NH₂, and R' is H, and acylating, if desired, to form a compound of Formula I wherein R' is acetyl, and, if desired, when R is NH—NH₂ (a) reacting said compound with a nitrosating agent to form an azide in which R is N₃ and then reacting said azide with ammonia, an amine of the formula HN(CH₃)₂, NH₂—alk—X, NH₂—(C₃—C₆)—cycloalkyl, NH₂—alk—Am or NH₂—alk—(OH)₁₋₃ wherein alk is (C₁—C₆)—alkylene and Am and X are as defined in Claim 1, to obtain

against certain tumors or classes of tumors and less effective against others.

- In utilizing the amides and hydrazides as anti-neoplastic agents, 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 except those in which R is NH—NH₂ or N₃, 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, the anti-neoplastic salt can be mixed with starch, a binder, and a lubricant and the mixture compressed into tablets each containing from 7.5—50 mgs. The tablets may be scored if lower or divided dosages are to be used. With parenteral administration, the intravenous route is preferred. For this purpose, isotonic solutions are employed containing 1—10 mg/ml of a salt of an indole-dihydroindole amide of Formula I except for the hydrazides and azides. The compounds are administered at the rate of from 0.1 to 1 mg/kg mammalian body weight once a week, depending on both the activity and the toxicity of the drug. Free bases of compounds according to Formula I in which R is NH—NH₂ or N₃, are compounded into suitable dosage forms and administered in similar fashion at similar dose levels.

- While most of the compounds are useful as anti-neoplastic or antiviral drugs, two types of derivatives, the hydrazides and azides (compounds of Formula I wherein R is NH—NH₂ or N₃), are also useful as intermediates as has been set forth above, in that the hydrazide can be transformed to the azide by reaction with a nitrosating agent, or to the simple amide by hydrogenolysis. The azide can in turn be made to react with primary or secondary amines to yield the amides of this invention.

- WHAT WE CLAIM IS:—
1. A compound of the formula



Formula I

wherein R is NH₂, NH—NH₂, N(CH₃)₂, NH—alk—X, NH(C₃—C₄)cycloalkyl, NH—alk—Am, NH—alk—(OH)₁₋₃, or N₃; wherein alk is (C₁—C₄) alkylene, Am is NH₂, NHCH₃, or N(CH₃)₂, and X is hydrogen, cyano, optionally substituted phenyl, carboxyl, carbo (C₁—C₃) alkoxy or carboxamide; wherein R' is H, (C₁—C₃) alkyl—CO or chloro—(C₁—C₃) alkyl—CO; wherein R'' is hydrogen, C₁—C₃ alkyl, formyl or (C₁—C₃) alkyl—CO; and wherein one of R''' and R'''' is hydroxyl and the other is ethyl; or a salt thereof.

2. A compound as claimed in claim 1, wherein R is NH₂, NH—NH₂, or NH—(C₁—C₃ alkyl); R' is (C₁—C₃) alkyl—CO or chloro (C₁—C₃) alkyl—CO; R'' is hydrogen, (C₁—C₃) alkyl or (C₁—C₃) alkyl—CO; R''' is OH and R'''' is ethyl.

3. A pharmaceutically-acceptable salt of a compound according to claim 1 or 2.

4. A sulfate salt of a compound according to claim 1, 2 or 3.

5. 4-Desacetyl vinblastine C—3 carboxamide.

6. 4-Desacetyl vinblastine C—3 carboxamide sulphate.

7. Vinblastine C—3 N—methylcarboxamide.

8. Vinblastine C—3 N—methylcarboxamide sulphate.

9. 4-Desacetyl leurocristine C—3 N-methylcarboxamide.

10. 4-Desacetyl leurocristine C—3 N-methyl carboxamide sulphate.

11. 4-Desacetyl vinblastine C—3 carboxhydrazide.

12. 4-Desacetyl vinblastine C—3 carboxazide.

13. 4-Desacetyl vinblastine C—3 carboxazide sulphate.

14. 4-Desacetyl vinblastine C—3 N-(2-hydroxyethyl) carboxamide.

15. A compound of Formula I, substantially as hereinbefore described with reference to any one of the foregoing Examples.

16. A process for preparing the compound of Formula I as defined in Claim 1 which comprises reacting a compound of Formula I wherein R is O—CH₃, R' is hydrogen or acetyl and R'', R''' and R'''' are as defined in Claim 1 with ammonia, methylamine or hydrazine to obtain a compound of Formula I wherein R is NH₂, NHCH₃, or NH—NH₂, and R' is H, and acylating, if desired, to form a compound of Formula I wherein R' is acetyl, and, if desired, when R is NH—NH₂, (a) reacting said compound with a nitrosating agent to form an azide in which R is N₃ and then reacting said azide with ammonia, an amine of the formula HN(CH₃)₂, NH₂—alk—X, NH₂—(C₃—C₄)—cycloalkyl, NH₂—alk—Am or NH₂—alk—(OH)₁₋₃, wherein alk is (C₁—C₄)—alkylene and Am and X are as defined in Claim 1, to obtain



NH₂, N(CH₃)₂,
cycloalkyl, NH—
(C₁—C₃)—, or N₃;
alkylene, Am is
and X is hydro-
substituted phenyl,
alkoxy, carbox-
(C₁—C₃)—alkyl—
yl—CO; where-
alkyl, formyl or
wherein one of
and the other is

as defined in claim 1,
or NH—(C₁—
alkyl—CO or
R' is hydrogen,
) alkyl—CO;

table salt of a
1 or 2.
and according to

C—3 carbox-

C—3 carbox-

-methylcarbox-

-methylcarbox-

—3 N-methyl-

ie C—3 N-

C—3 carbox-

C—3 carbox-

C—3 carbox-

C—3 N-(2-

, substantially
reference to
les.

the compound
aim 1 which

of Formula
hydrogen or

as defined
ethylamine or

of Formula I

NH—NH₂,
ired, to form
R' is acetyl,
[—NH₂ (a)

nitrosating
R is N₃ and

ammonia, an
HN(CH₃)₂,
—cycloalkyl,

—(OH)₁₋₃

ne and Am
1, to obtain

the corresponding compound of Formula I,
or (b) hydrogenolyzing said compound in
which R is NH—NH₂ with Raney nickel in
an inert solvent to yield a compound of
Formula I wherein R is NH₂, R' is hydrogen
or acetyl, and R'', R''' and R'''' are as defined
in claim 1.

17. A process as in claim 16 which com-
prises the additional step of acylating the
product of claim 16 wherein R' is hydrogen
to produce the corresponding product in
which R' is (C₁—C₃)—alkyl—CO or
chloro—(C₁—C₃)—alkyl—CO, and, if in the
said product of claim 16 R'' is hydrogen, in
which R'' is (C₁—C₃)—alkyl—CO or
chloro—(C₁—C₃)—alkyl—CO.

18. A process for the preparation of a
pharmaceutically acceptable acid addition salt
of the product of claim 16 or 17 which
comprises reacting said product with a non-
toxic inorganic acid or organic acid.

19. A process as in claim 18 wherein the
inorganic acid is hydrochloric acid, nitric acid,
orthophosphoric acid, sulfuric acid, hydro-
bromic acid, hydroiodic acid, nitrous acid or
phosphorous acid.

20. A process as in claim 18 wherein the
organic acid is an aliphatic mono- or di-
carboxylic acid, phenyl-substituted alkanic
acid, hydroxy alkanic acid, hydroxyalkandioic
acid, aromatic carboxylic acid, aliphatic
sulfonic acid or aromatic sulfonic acid.

21. A process for the preparation of 4-des-
acetyl vinblastine C—3 N-methylcarboxamide
which comprises reacting vinblastine with
methylamine in the presence of methanol at
a reduced temperature to obtain a mixture
of 4-desacetyl vinblastine C—3 N-methyl-
carboxamide and 4-desacetyl vinblastine, and
recovering 4-desacetyl vinblastine C—3 N-
methylcarboxamide.

22. A process as in claim 21 which com-
prises the additional step of reacting 4-des-
acetyl vinblastine C—3 N-methylcarboxamide
with sulfuric acid to obtain 4-desacetyl vin-
blastine C—3 N-methylcarboxamide sulfate.

23. A process for preparing 4-desacetyl
vinblastine C—3 carboxamide which com-
prises reacting vinblastine with ammonia in
the presence of an inert solvent at an elevated
temperature.

24. A process for preparing 4-desacetyl
vinblastine C—3 carboxhydrazide which com-
prises reacting 4-desacetyl vinblastine with
anhydrous hydrazine in the presence of an-
hydrous ethanol at an elevated temperature.

25. A process for preparing 4-desacetyl
1-desformyl leurocristine C—3 carbox-
hydrazide which comprises reacting 4-des-
acetyl leurocristine with anhydrous hydrazine
in the presence of anhydrous methanol at an
elevated temperature.

26. A process for preparing 4-desacetyl
leurocristine C—3 N-methylcarboxamide
which comprises saturating a solution of leuro-

cristine and methanol with gaseous hydrogen
chloride at a reduced temperature, allowing
reaction to proceed at ambient temperature,
dissolving the resulting residue in water, add-
ing ammonium hydroxide to form an alkali
insoluble base, extracting said base with
methylene chloride, evaporating to dryness to
obtain 4-desacetyl 1-desformyl leurocristine,
reacting 4-desacetyl 1-desformyl leurocristine
with methylamine in the presence of methanol
at an elevated temperature, and reacting the
resulting 4-desacetyl 1-desformyl leurocristine
C—3 N-methylcarboxamide with formic acid
and acetic anhydride.

27. A process for preparing 4-desacetyl
vinblastine C—3 carboxazide which comprises
reacting 4-desacetyl vinblastine C—3 carbox-
hydrazide with sodium nitrite at a reduced
temperature in the presence of methanol and
hydrochloric acid.

28. A process for preparing 4-desacetyl
vinblastine C—3 N-ethylcarboxamide which
comprises reacting 4-desacetyl vinblastine
C—3 carboxazide with anhydrous ethylamine
in the presence of methylene dichloride.

29. A process for preparing 4-desacetyl
vinblastine C—3 N-isopropylcarboxamide
which comprises reacting 4-desacetyl vin-
blastine C—3 carboxazide with anhydrous
isopropylamine in the presence of methylene
dichloride.

30. A process for preparing 4-desacetyl
vinblastine C—3 N,N-dimethylcarboxamide
which comprises reacting 4-desacetyl vin-
blastine C—3 carboxazide with anhydrous
N,N-dimethylamine in the presence of
methylene dichloride.

31. A process for preparing 4-desacetyl
vinblastine C—3 N[2-(N,N-dimethylamino)-
ethyl]carboxamide which comprises reacting
4-desacetyl vinblastine C—3 carboxazide and
N,N-dimethyl ethylenediamine in the presence
of methylene dichloride.

32. A process for preparing 4-desacetyl
vinblastine C—3 N-benzylcarboxamide which
comprises reacting 4-desacetyl vinblastine
C—3 carboxazide with benzylamine in the
presence of methylene dichloride.

33. A process for preparing 4-desacetyl
vinblastine C—3 N-cyanomethylcarboxamide
which comprises reacting 4-desacetyl vin-
blastine C—3 carboxazide with cyanomethyl-
amine in the presence of methylene dichloride.

34. A process for preparing 1-desformyl
4-desacetyl leurocristine C—3 N-ethyl-
carboxamide which comprises reacting 1-des-
formyl 4-desacetyl leurocristine C—3 carbox-
hydrazide with sodium nitrite in dilute hydro-
chloric acid at a reduced temperature to form
1-desformyl 4-desacetyl leurocristine C—3
carboxazide and reacting said carboxazide with
ethylamine in the presence of methylene di-
chloride.

35. A process for preparing 4-desacetyl
leurosidine C—3 amide which comprises react-



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ing leucosidine with anhydrous hydrazine in anhydrous methanol at an elevated temperature, reacting the resulting 4-desacetyl leucosidine C—3 carboxhydrazide with sodium nitrite at a reduced temperature, and reacting the resulting azide at a reduced temperature with methanol saturated with ammonia.

36. A process of preparing a compound of Formula 1 or a salt thereof, substantially as hereinbefore described with reference to any one of the foregoing Examples.

37. A compound of Formula I whenever prepared by a process according to any one of claims 16 to 35.

38. A pharmaceutical formulation comprising a compound of Formula I or a pharmaceutically acceptable salt thereof as claimed in any one of claims 1 to 15 or 37 in association with a pharmaceutically acceptable carrier therefor.

39. A pharmaceutical preparation in dosage unit form adapted for administration to obtain an anti-neoplastic effect, comprising per dosage unit an effective amount within the range 0.1 to 1.0 mg/kg of mammalian body weight of a salt according to claim 3 plus a pharmaceutical diluent.

40. The method of treating neoplasms which comprises administering to non-human mammal suffering from a neoplastic disease an

anti-neoplastically-effective amount in the range of 0.1 to 1.0 mg/kg of mammalian body weight of a salt according to claim 3.

41. A pharmaceutical preparation in unit dosage form adapted for administration to obtain an anti-neoplastic effect, comprising per dosage unit an effective amount within the range 0.1—1.0 mg/kg of mammalian body weight of a compound according to claim 1 in which R is NH—NH₂ and R', R'', R''' and R'''' are as defined in claim 1.

42. A method of treating neoplasms which comprises administering to a non-human mammal suffering from an neoplastic disease an anti-neoplastically-effective amount in the range 0.1 to 1.0 mg/kg of mammalian body weight of a compound according to claim 1 in which R is NH—NH₂ and R', R'', R''' and R'''' are as defined in claim 1.

43. A pharmaceutical formulation comprising a compound of Formula I or a pharmaceutically acceptable salt thereof substantially as herein described.

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