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NOVEL AMINOACYL ESTERS OF DESACETYL VINCALEUKOBLASTINE

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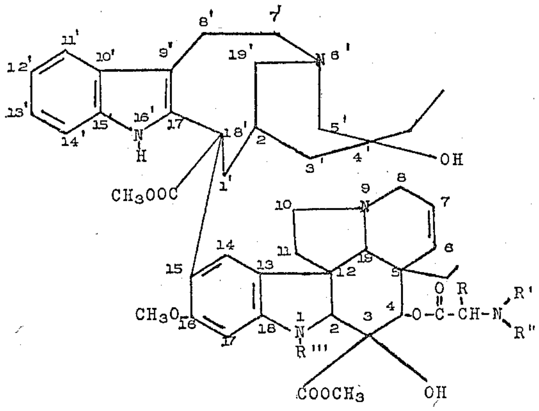
ABSTRACT OF THE DISCLOSURE

α-Substituted-α-aminoacylated esters of certain desacylated polynuclear indoles are provided. These compounds are useful in affecting the growth of transplanted tumor cells in host animals.

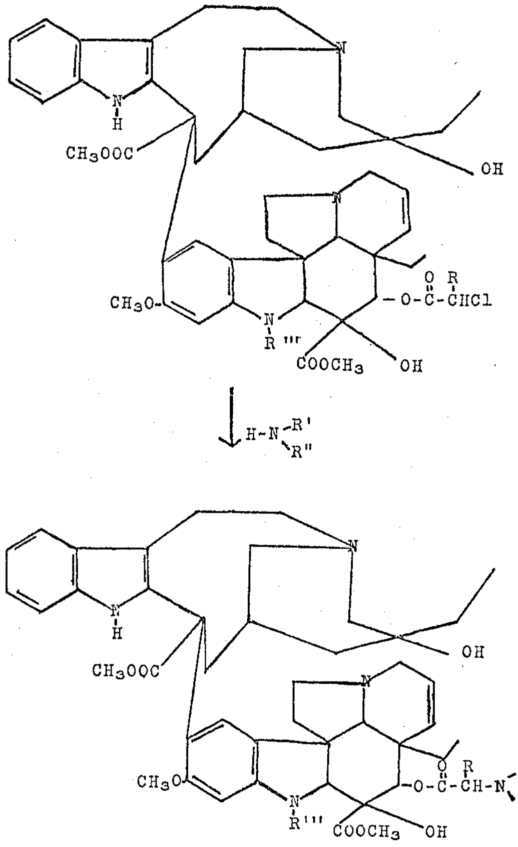
This invention relates to novel α-substituted-α-aminoacylated esters of certain desacylated polynuclear indoles, and to novel procedures useful in the preparation of such esters.

The compounds provided by this invention can be represented by the following formula:



wherein R is a member of the group consisting of hydrogen, C_1–C_5 alkyl, C_3–C_8 cycloalkyl, monocyclic aryl, and bicyclic aryl; R′, when taken alone, is a member of the group consisting of hydrogen, C_1–C_5 alkyl, and C_3–C_8 cycloalkyl; R″, when taken alone, is C_1–C_5 alkyl; R′ and R″, when taken together with the nitrogen atom to which they are attached, form a member of the group consisting of pyrrolidino, piperidino, piperazino, morpholino, 4-(C_1–C_5 alkyl)-piperazino, 4-(hydroxy-substituted C_1–C_5 alkyl)piperazino, and 4-(monocyclic aryl)piperazino; and R‴ is a member of the group consisting of H-CO-, C_1–C_5 alkyl, and C_1–C_5 alkyl-CO—. Non-toxic salts of the above amines are also included within the scope of this invention.

Compounds represented by the above formula are prepared according to the procedure graphically formulated below:



wherein R, R′, R″ and R‴ have the same meaning as hereinabove.

In the above formulas, when R, R′, R″ or R‴ represent C_1–C_5 alkyl groups, they can represent, illustratively, methyl, ethyl, isopropyl, n-propyl, n-butyl, isobutyl, sec.-butyl, t-butyl, n-amyl, t-amyl, neopentyl, 2-pentyl, 3-pentyl, and 3-methyl-2-butyl. When R or R′ represents a C_3–C_8 cycloalkyl group, they can be illustratively cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, or cyclo-octyl. When R‴ represents C_1–C_5 alkyl-CO, any of the above groups used to illustrate C_1–C_5 alkyl may be attached to the CO radical, thus yielding such typical acyl groups as acetyl, propionyl, butyryl, isobutyryl, trimethylacetyl, and the like. When R represents monocyclic or bicyclic aryl, the term "aryl" includes all those monocyclic and bicyclic radicals satisfying the usual criteria for aromaticity including phenyl, naphthyl, pyridyl, pyrimidyl, pyrazinyl, pyridazinyl, thienyl, furyl, indanyl, thianaphthyl, quinolinyl, isoquinolinyl, pteridyl, and the like. Also included within the scope of the term "aryl," as used in the instant disclosure, are aryl groups containing conventional substituents in any of the available positions of the aryl ring, such substituents including halogens such as fluorine, chlorine, bromine, and iodine; C_1–C_5 alkyl; C_2–C_5 alkenyl, including allyl, methallyl, crotyl, and the like; amino; C_1–C_5 alkylamino; di-(C_1–C_5 alkylamino; halo-substituted lower alkyl wherein the halogens are those listed above, such groups to include trifluoromethyl, pentafluoroethyl, β-chloroethyl, trichloromethyl, and the like; cyano; carboxyl; carboxy C_1–C_5 alkyl; C_1–C_5 alkoxy; etc. In all the above groupings, "C_1–C_5 alkyl" includes the same radicals listed above for purposes of illustrating the scope of that term for R, R', R'' and R'''.

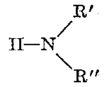
When R' and R'', together with the nitrogen atom to which they are attached, form a piperazine ring bearing a substituent other than hydrogen in the 4-position, the 4-(C_1–C_5 alkyl)-piperazines, the 4-(hydroxy-substituted C_1–C_5 alkyl)piperazines and the 4-(monocyclic aryl)piperazines are set forth specifically as illustrative of these 4-substituted piperazines. The terms "C_1–C_5 alkyl" and "monocyclic aryl" as employed herein include the same radicals as those employed above to illustrate the terms in conjunction with definitions of R, R', R'' or R'''. The term "hydroxy-substituted C_1–C_5 alkyl" includes all of the above C_1–C_5 alkyl groups when substituted with hydroxy at any available position of the alkyl chain, including such groups as 2-hydroxyethyl, 2-hydroxypropyl, 3-hydroxy-2-pentyl, 2-hydroxy-1,1-dimethylethyl and the like.

As regards the generic nomenclature of compounds represented by Formula I above, when R''' is methyl, the compounds belong to the vincaleukoblastine (vinblastine) series and are esters of desacetyl vincaleukoblastine, to be referred to hereinafter as desacetyl VLB. In Formula I above, when R''' is formyl, the compounds thus represented belong to the leurocristine (vincristine) series and are referred to as esters of desacetyl leurocristine, to be referred to hereinafter as desacetyl LCR. Compounds represented by Formula II above in which R''' is other than methyl or formyl do not correspond to any known alkaloid, and there is no generic name available to describe them. They are prepared by alkylating or acylating desmethyl desacetyl VLB chloroacetate with an appropriate alkylating or acylating agent. For example, desmethyl desacetyl VLB chloroacetate can be reacted with acetic anhydride at room temperature to yield N-acetyl desmethyl desacetyl VLB chloroacetate. Other acylating agents such as propionic anhydride, butyric anhydride or mixed anhydrides of trifluoro and stearic acid can be used to prepare the corresponding N-acyl derivative in like fashion. Similarly, desmethyl desacetyl VLB chloroacetate can be reacted with acetaldehyde in the presence of hydrogen and a platinum catalyst to yield N-ethyl desmethyl desacetyl VLB chloroacetate. Other common alkylating reagents can be used to produce higher homologs of the above compounds, as for example the N-n-propyl, the N-n-butyl, the N-isobutyl, the N-isoamyl and the like derivatives.

Also included within the scope of the invention are the salts of the dimeric indole alkaloids represented by Formula I above formed with non-toxic acids. Included among such non-toxic acids are hydrochloric acid, sulfuric acid, phosphoric acid, phosphorus acid, boric acid, acetic acid, benzoic acid, succinic acid, maleic acid, tartaric acid, citric acid, and the like. Because of the ease of hydrolysis of various groupings in the dimeric indoles of this invention, the preparation of salts must be undertaken with considerably more care than is customary with conventional organic bases. For example, the sulfate salt of one of the compounds of this invention can be prepared by dissolving the free base in aqueous methanol or in ethanol, and then adding to this solution an equivalent amount of a 1% solution of sulfuric acid in the same solvent. Where an aqueous solvent system is being employed, the dilute acid can be added until a predetermined pH has been reached. The acid addition salt is then isolated by evaporation of the solvents in vacuo, taking care to avoid any excess heating.

Other non-toxic salts can be prepared by adapting the above procedure to a particular acid, taking care to avoid any conditions which might result in hydrolysis of one or more of the ester groups present in the dimetric indole alkaloid.

According to the above equation, desacetyl VLB chloroacetate, or desacetyl LCR chloroacetate, or a chloroacetate of a desacetyl compound in which R''' is other than methyl or formyl (the preparation of these chloroacetates is set forth in my copending application, Ser. No. 350,519, filed Mar. 9, 1964) is reacted with an excess of a primary or secondary amine, represented by the formula



$$H-N\begin{matrix}R'\\ R''\end{matrix}$$

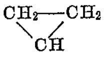
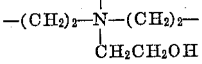
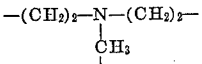
in an inert solvent, such as, for example, tetrahydrofuran, carbon tetrachloride, ethanol, ethyl acetate, and benzene. During this reaction, the hydrochloride salt of the amine precipitates and is removed by filtration. The thus formed desacetyl VLB aminoacetate or desacetyl LCR aminoacetate or similar aminoacetate is isolated by conventional procedures involving, for example, dissolution of the desired compound in an acidic aqueous phase, extraction of the acidic aqueous phase with a water-immiscible solvent, basification of the aqueous phase, followed by extraction of the novel alkaloidal compound into a water-immiscible organic solvent, separation of the organic layer, and recovery of the desired compound as a residue after evaporation of the solvent. The novel alkaloid derivative thus obtained is further purified either by chromatography or by converting the free alkaloid base into a mineral acid salt, and recrystallizing this salt or by other suitable process. Another procedure which is available for the purification of the free bases prepared by the procedures outlined above is a gradient pH extraction procedure following the method set forth in the copending application of Gordon Svoboda et al., Ser. No. 147,911, filed Oct. 26, 1961, now U.S. Patent No. 3,205,220.

Illustrative compounds which can be prepared according to the above procedure include the following:

N,N-dimethyl desacetyl VLB α-phenylglycinate,
N,N-diethyl desacetyl LCR glycinate,
N-methyl-N-isopropyl 1-ethyl desmethyl desacetyl VLB glycinate,
N,N-di-n-amyl desacetyl VLB α-cyclopropyl glycinate,
N-t-amyl 1-acetyl desmethyl desacetyl VLB leucinate,
N-t-butyl desacetyl LCR α-(2-naphthyl) glycinate,
N-methyl-N-isopropyl 1-isobutyryl desmethyl desacetyl VLB α-cyclohexyl glycinate,
N-cyclo-octyl 1-valeryl desmethyl desacetyl VLB tyrosinate, desacetyl VLB α-morpholinoacetate,
1-(3-methyl-2-butyl) desmethyl desacetyl VLB α-(4-methylpiperazino)tyrosinate,
desacetyl VLB α-(chlorophenyl)-α-piperidinoacetate,
desacetyl VLB α-pyrrolidinoacetate, and the like.

The compounds of this invention have utility in the study of the mechanism by which cancer and related malignancies attack an animal host. For example, the compounds of this invention greatly prolong the life of DBA/2 mice inoculated with a lethal dose of p–1534 leukemia cells. The following table gives the results of tests in which the compounds of this invention demonstrated this desirable utility. In the table, column 1 gives the R substituent from Formula I, column 2 the R' substituent, column 3 the R'' substituent, column 4 the R''' substituent, column 5 the dose level, and column 6 the percent prolongation of life in the treated mice over that of a control group of mice injected only with the particular pharmaceutical vehicle used for the injections of the active drug.

TABLE I

R	R′	R″	R‴	Dose Level in mg./kg.	Percent Prolongation
H	CH_3	CH_3	CH_3	5	5 out of 5 survivors.
H	$-(CH_2)_4-$		CH_3	7.5	100%.
H	$-(CH_2)_5-$		CH_3	3	24%.
H	$-(CH_2)_2-O-(CH_2)_2-$		CH_3	15	127%.
H	CH_3		CH_3	3.75	201%+4 out of 5 survivors.
H	 $-(CH_2)_2-N(CH_2CH_2OH)-(CH_2)_2-$		CH_3	15	78%.
H	 $-(CH_2)_2-N(CH_3)-(CH_2)_2-$		CH_3	15	30%.
H	CH_3	H	CH_3	3	187%+3 out of 5 survivors.

The compounds of this invention are closely related to the proven oncolytic agents, vinblastine and vincristine, as well as to the experimentally useful alkaloids, vinleurosine and vinrosidine. The compounds of this invention, providing as they do a wide range of possible substituents which replace an acetyl group in the vinblastine or vincristine molecule, are extremely valuable agents for the study of the mechanism of action of these alkaloidal drugs on various leukemias and lymphomas since, for the first time, it will be possible to have structure-activity relationships determined in this interesting group of compounds.

This invention is further illustrated by the following specific examples:

Example I.—N,N-dimethyl desacetyl VLB glycinate

Five hundred milligrams of desacetyl VLB chloroacetate were dissolved in 10 ml. of tetrahydrofuran. Four milliliters of a 25 percent solution of dimethylamine in tetrahydrofuran were added, and the resulting mixture was allowed to remain overnight at room temperature. A precipitate of dimethylamine hydrochloride, produced as a by-product of the reaction, was removed by filtration and the filtrate, containing N,N-dimethyl desacetyl VLB glycinate, was evaporated to dryness in vacuo. Fifty milliliters of water were added to the residue, and the aqueous solution was made basic by the addition of an excess of 14 N ammonium hydroxide. N,N-dimethyl desacetyl VLB glycinate was insoluble in the alkaline layer and was extracted into methylene dichloride. The methylene dichloride layer was separated and dried, and the solvent removed therefrom by evaporation in vacuo. The residue, comprising N,N-dimethyl desacetyl VLB glycinate, was crystallized from ether. Thin layer chromatography on alumina indicated that this product was pure and differed from the starting material. Infrared and nuclear magnetic resonance spectra of the crystalline material were obtained, and these spectral data were completely consistent with the assigned structure.

Crystalline N,N-dimethyl desacetyl VLB glycinate was dissolved in a mixture of methanol and water. The pH of the solution was adjusted to about 1.8 with 1 percent sulfuric acid. Evaporation of the resulting solution to drynes in vacuo yielded N,N-dimethyl desacetyl VLB glycinate sulfate as a residue. The residue was crystallized from a mixture of methanol and ethanol. Nuclear magnetic resonance and infrared spectra of the sulfate salt were entirely consistent with the assigned structure.

N,N-diethyl desacetyl VLB glycinate was prepared by substituting diethylamine for dimethylamine in the above procedure.

N-ethyl-N-N-propyl desacetyl VLB glycinate was prepared by substituting N-ethyl n-propylamine for dimethylamine in the above procedure.

Example II.—Desacetyl VLB α-(4-phenylpiperazino) acetate

Following the procedure of Example I, desacetyl VLB chloroacetate was reacted with 4-phenylpiperazine to yield desacetyl VLB α-(4-phenylpiperazino)acetate. The latter compound was crystallized from a mixture of methylene dichloride and ether, and the crystalline product was chromatographed over grade II alumina. Chromatographic fractions which showed the presence of a single spot on thin layer chromatography over alumina were combined and again crystallized from a mixture of methylene dichloride and ether.

The sulfate salt of desacetyl VLB α(4-phenylpiperazino)acetate was prepared by the method of Example I and yielded crystalline material from a mixture of methanol and ethanol.

Desacetyl VLB α-piperazinoacetate was prepared in the same fashion by reacting piperazine and desacetyl VLB chloroacetate. The compound was isolated and purified by the procedure of Example I, and the corresponding sulfate salt was made by the procedure of the same example. Desacetyl VLB α-piperazinoacetate sulfate thus prepared was recrystallized from ethanol.

Desacetyl VLB α-[4-(β-hydroxyethyl)piperazino]acetate was prepared in the same fashion by reacting 4-(β-hydroxyethyl)piperazine and desacetyl VLB chloroacetate. The reaction product yielded only a single spot on thin layer chromatography. A crystalline sulfate was also prepared by the procedure of Example I.

Desacetyl VLB α-(4-methylpiperazino)acetate was prepared by reacting 4-methylpiperazine with desacetyl VLB chloroacetate. Thin layer chromatography showed the product of the reaction to be a single substance. A water-soluble, crystalline sulfate salt of desacetyl VLB α-(4-methylpiperazino)acetate was prepared by the procedure of Example I.

Example III.—N-methyl-N-cyclopropyl desacetyl VLB glycinate

Desacetyl VLB chloroacetate was reacted with N-methyl cyclopropylamine by the procedure of Example I, except that the reaction mixture was allowed to remain at room temperature for about 48 hours, and at the end of that time was heated for about one hour at 50° C. The product was isolated by the procedure of Example I and was chromatographed over a grade II alumina column using 9:1 benzene-chloroform as the eluant. The first fractions from the chromatographic column were combined and evaporated to dryness in vacuo. A nuclear magnetic resonance spectrum of an aliquot of the resulting residue indicated that the desired product had been formed and was present in both fractions. The sulfate salt of N-methyl-N-cyclopropyl desacetyl VLB glycinate thus prepared was formed by dissolving N-methyl-N-cyclopropyl desacetyl VLB glycinate in ethanol and adding 1 percent ethanolic sulfuric acid until the pH of the original solution had been decreased to about 3.5. Evaporation of the solvents in vacuo yielded a residue of N-methyl-N-cyclopropyl desacetyl VLB glycinate sulfate which was crystallized from a mixture of methanol and ethanol.

Example IV.—Desacetyl VLB α-morpholinoacetate

Following the procedure of Example I, desacetyl VLB chloroacetate was reacted with morpholine in tetrahydrofuran solution. Desacetyl VLB α-morpholinoacetate was isolated and purified by the process of Example I. Infrared and nuclear magnetic resonance spectra of the resulting product were entirely consistent with the proposed structure. The corresponding sulfate salt was prepared by the process of Example I.

Example V.—Desacetyl VLB α-piperidinoacetate

Following the procedure of Example I, desacetyl VLB chloroacetate was reacted with piperidine in tetrahydrofuran solution. Desacetyl VLB α-piperidinoacetate thus prepared was isolated and purified by the procedure of Example I, and gave only one spot on thin layer chromatography, thus demonstrating that only a single substance was present. The corresponding sulfate salt was prepared by the procedure of Example I.

Example VI.—Desacetyl VLB α-pyrrolidinoacetate

Following the procedure of Example I, desacetyl VLB chloroacetate was reacted with pyrrolidine in tetrahydrofuran solution to yield desacetyl VLB α-pyrrolidinoacetate, which was separated and purified by the procedure of the same example. Desacetyl VLB α-pyrrolidinoacetate thus prepared was recrystallized from benzene. Infrared and nuclear magnetic resonance spectra of the crystalline product were entirely consistent with the assigned structure. A crystalline sulfate salt was prepared by the procedure of Example I.

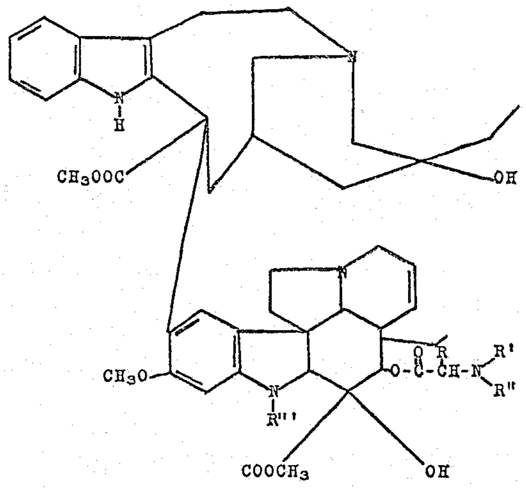
Example VII.—N-methyl desacetyl VLB glycinate

Following the procedure of Example I, desacetyl VLB chloroacetate and N-methylamine were reacted in tetrahydrofuran solution to yield N-methyl desacetyl VLB glycinate which was isolated by the procedure of Example I. The material thus obtained was submitted to a gradient pH extraction procedure as set forth in the copending application of Gordon Svoboda et al., Ser. No. 147,911, filed Oct. 26, 1961. Fractions extracted at pH 6.0, 6.8, and 7.6 were combined and were chromatographed over alumina, using benzene as an eluant. Fractions from this chromatographic procedure, yielding only a single spot on this layer chromatography, were combined. N-methyl desacetyl VLB glycinate was crystallized from these fractions using a mixture of methylene dichloride and ether as the crystallization solvent.

The corresponding sulfate salt was prepared by the method of Example I, but was not crystallized.

I claim:

1. A compound of the formula



wherein R is a member of the group consisting of hydrogen and C_1–C_5 alkyl; R', when taken alone, is methyl or cyclopropyl; R'', when taken alone, is methyl, cyclopropyl or hydrogen; R' and R'', when taken together with the nitrogen atom to which they are attached, form a member of the group consisting of pyrrolidino, piperidino, morpholino, 4-methylpiperazino, and 4-(β-hydroxyethyl) piperazino, and R''' is methyl; and salts thereof with nontoxic acids.

2. N,N-dimethyl desacetyl vincaleukoblastine glycinate.

3. N-methyl-N-cyclopropyl desacetyl vincaleukoblastine glycinate.

4. N-methyl desacetyl vincaleukoblastine glycinate.

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