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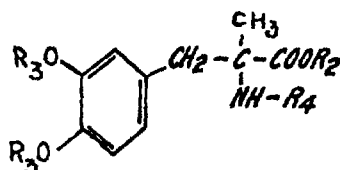
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International Classification:—C07c, d

COMPLETE SPECIFICATION

α -Amino Acid Derivatives related to L-Phenylalanine

- We, MERCK & CO., INC., a corporation duly organised and existing under the laws of the State of New Jersey, United States of America, of Rahway, New Jersey, 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 amino acids some of which are useful as antihypertensive agents. In brief, this invention provides compounds of the formula:



- in which, R_2 is a hydrogen atom or an alkyl radical and each of R_3 and R_4 is a hydrogen atom or a C_{1-6} alkanoyl radical, the said compounds being in the *L* or sinister absolute spatial configuration at the α -carbon atom, i.e. the same spatial configuration as *L*-dihydroxyphenylalanine, and substantially free, i.e., containing not more than about 5%, of the *D* or rectus enantiomorphs, as well as the non-toxic salts of such compounds.

- The DL racemic form of α - methyl - β -

[Price 4s. 6d.]

(3,4 - dihydroxyphenyl) alanine is described in the literature, (see, for Example, U.S. Patent No. 2868818). This compound has now been found by thorough clinical testing to be a very valuable antihypertensive agent. It has been found especially valuable in those cases of hypertension where kidney damage has also occurred. It is also especially useful in cases of a malignant hypertension. Furthermore, experimental work has demonstrated that many of its alkyl esters, due to enzymatic saponification in VIVO share with this compound the properties which make it so useful therapeutically.

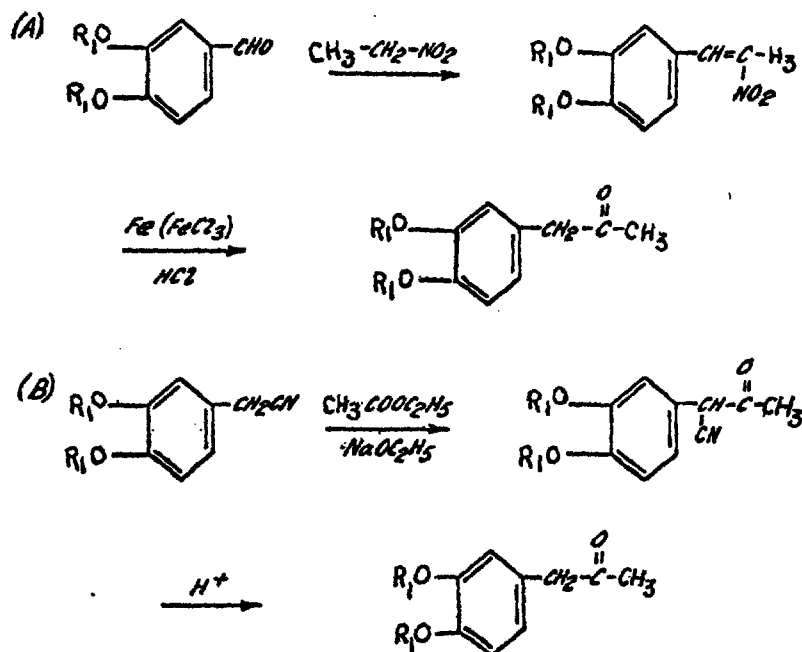
It has been found that the active compounds of the class defined above have the therapeutic activity solely in the *L* or sinister spatial configuration and that, consequently, the *L* form of this class of compounds, substantially free of the *D* or rectus spatial configuration, is more potent and less toxic than the racemic mixture. In this configuration the removal of the *D* form from the racemic mixture is not merely the removal of an inert ingredient, for while the *D* form is therapeutically inert as an anti-hypertensive agent, it is just as toxic as the *L* form. Consequently, its removal is important in providing a safer drug. The therapeutic index (usually defined as the therapeutic dose divided by the toxic dose) is thus greatly decreased by removal of the *D* form.

It is most surprising to find that all the therapeutic activity resides in the *L* form although the *D* form is still as toxic as the *L* form. Toxicity is a form of biological activity and compounds which show biological activity usually have toxicity associated with it. In this case, both of the enantiomorph forms of

the above class have the identical toxicity but all the therapeutic activity resides solely in the *L* form.

In the synthesis of the compounds of the invention, the starting material is a ketone whose preparation is shown in the following reaction scheme:

Scheme I



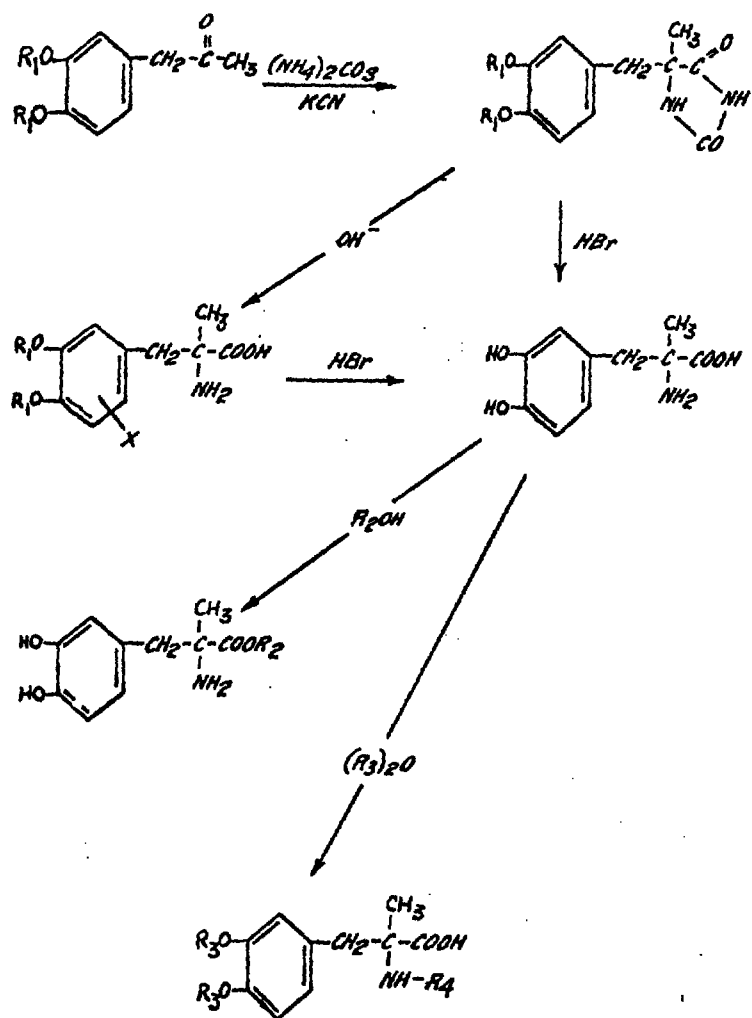
where each of R_1 is a hydrogen atom or a methyl radical, or both R_1 groups together represent a methylenedioxy group, provided that the R_1 groups are not both hydrogen atoms.

With reference to this reaction scheme, it can be seen that there are two general syntheses for the starting ketones, the first from a substituted benzaldehyde by reaction with a nitroethane and the second from a substituted phenylacetonitrile by a Claisen condensation, followed by ketone splitting. It should be noted in the general formulae of the starting materials and of the ketones that normally dimethoxy substituted phenyl radicals are used

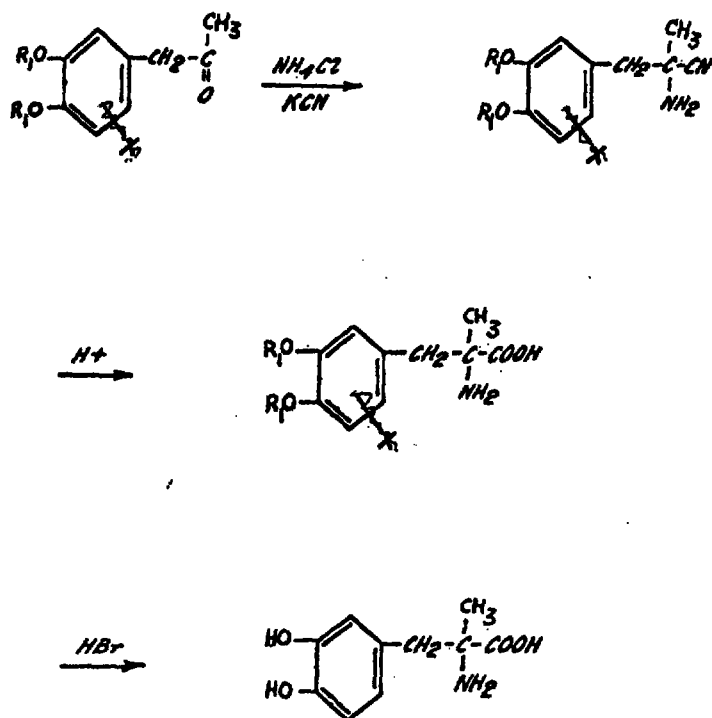
but that one hydroxyl may be present with one methoxy radical. It is not advisable to have more than one free hydroxyl because of the sensitivity of these structures to oxidation.

The racemic amino acids are prepared by two standard methods which are shown in detail in the following reaction schemes II and III. In reaction scheme III, the synthesis has been stopped at the free hydroxyphenylamino acid stage but the further step of esterification, of the carboxy group can be carried out as in reaction scheme II. It should be noted that, in the final compound in Scheme II, R_1 and R_2 are the same C_{1-6} alkanoyl radicals.

Scheme II



Scheme II

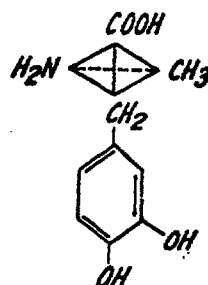


Of the two procedures, the preferred procedure is that shown in Scheme II. It involves heating the starting ketone with ammonium carbonate and a water-soluble cyanide salt in aqueous solution to form a hydantoin derivative. This hydantoin derivative can be directly hydrolysed by a hydrohalic acid to the free hydroxyphenylamino acid. Alternatively, the hydantoin ring may be split with a base, e.g. barium hydroxide or sodium or potassium hydroxide, to give the methoxyphenyl amino acid structure which, in turn, upon treatment with a hydrohalic acid, gives the hydroxyphenylamino acid. As indicated in the reaction scheme, by proper treatment with an alcohol, esters of the free amino acid in which the carboxy is esterified can be prepared, and by treatment with an alkanolic anhydride, the ring hydroxyls and amino nitrogen can be simultaneously acylated.

The alternative synthesis requires the condensation of the starting ketone with ammonium chloride and a water-soluble cyanide salt to form an aminonitrile. Acid hydrolysis of this converts the nitrile group first to the amide, and then to the free amino acid, and this, upon further treatment with a hydrohalic acid, gives the hydroxyphenylamino acids.

The above chemical synthesis produces the

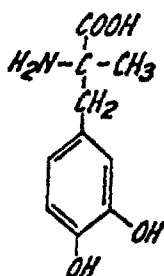
racemic mixtures. In order to produce the *L* form free of the *D* form as required by the invention, it is necessary to resolve the racemic mixture at some point in or after the synthesis. It has been found by comparison of the rotatory dispersion curve with an authentic sample of *D*(+)-dihydroxyphenylalanine and the known curve for its *L* isomer that the laevorotatory form at the *D* line of α -methyl- β -(3,4-dihydroxyphenyl)alanine — the form in which all the therapeutic activity resides — belongs to the *L* or sinister spatial configuration family. Such a spatial arrangement may be described by:



in which the tetrahedron represents the asymmetric α carbon. It is also often written:—

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Condensation of the starting ketone to form either the amino nitrile or the hydantoin results in formation of the asymmetric α -carbon and resolution of the racemic mixture at an early stage in the synthesis has the advantage of permitting the recycling of the intermediate by regeneration of the starting ketone from the discarded *D* form.

In accordance with the present invention, chemical resolution is effected by forming diastereomers from the racemic mixture by reaction with an optically active resolving agent. Thus, in a preferred embodiment of the invention, an optically active base is reacted with the carboxyl group, with or without blocking (e.g. by acylation) of the amino groups. As an alternative, which is less suitable, an optically active acid can be reacted with the amino group with or without blocking of the carboxy, as for example, by esterification, amide formation, or the use of the nitrile at an early stage in the synthesis. The difference is solubility between the diastereomers formed permits the selective crystallization of one form and regeneration of the optically active amino acid from the mixture.

Another method of resolving viz, selective enzymatic reaction, shows promise. Thus the racemic amino acid can be subjected to an asymmetric oxidase or decarboxylase which will, by oxidation or decarboxylation, destroy one form, usually the *L*, leaving the other form unchanged. Even more attractive is the use of a hydrolase on a derivative of the racemic mixture to form preferentially one form of the amino acid. Thus carboxy esters or carboxamides of the amino acids can be subjected to an esterase which will selectively hydrolyse one enantiomorph and leave the other unchanged. Similarly, the *N*-acyl derivatives may be subjected to a hydrolase which will selectively deacylate one enantiomorph and leave the enantiomorph amide unchanged.

In practice, it has been preferred to carry out the resolution upon the final racemic amino acid, usually in the form of an *N*-acyl derivative. Two alternative methods may be used. Either the intermediate methoxyphenylamino acid can be acylated and resolved or the free racemic hydroxyphenylamino acid is peracylated to give an acyloxy phenyl *N*-acylamino acid and this is resolved. In both cases,

the resolution is carried out by the formation of salts with optically active bases. Such bases may include such compounds as quinine, brucine, cinchonidine, cinchonine, hydroxyhydrindamine, methylamine, morphine, α -phenylethylamine, phenyloxynaphthylmethylamine, quinidine, 1-fenchylamine, strychnine, basic amino acids such as lysine and arginine, and amino acid esters.

In the method of resolving the amino esters by making salts with optically active acids, one may use such acids as α -bromocamphor- π -sulphonic acid, camphoric acid, camphor-10-sulphonic acid, helicic acid, malic acid, mandelic acid, menthoxyacetic acid, oxyethylene camphor, quinic acid, tartaric acid, 2,2'-dinitrodiphenic acid, and diacyltartaric acid, and diacylglutamic acids.

Among the compounds which form the invention apart from *L*- α -methyl- β -(3,4-dihydroxyphenyl)alanine, are its alkyl carboxy esters, such as methyl, ethyl, isopropyl, butyl, lauryl and octadecyl esters and the lower alkanoyl derivatives such as the formyl, acetyl, propionyl and the like derivatives, of the *O*-acyl *N*-acyl type. When a demethylation step is necessary, the amino group can be acylated before demethylation of the nuclear methoxy groups.

In accordance with a practical embodiment of the present invention, *L*- α -methyl- β -(3,4-dihydroxyphenyl)alanine is prepared substantially free of its *D*-enantiomorph by reacting *DL*- α -methyl- β -(3,4-dimethoxyphenyl)alanine with acetic anhydride in the presence of a proton acceptor, thereby forming *DL*-*N*-acetyl- α -methyl- β -(3,4-dimethoxyphenyl)alanine, reacting this compound with an optically active *L*- α -phenylethylamine in an organic solvent at elevated temperature, recovering crystalline *L*-*N*-acetyl- α -methyl- β -(3,4-dimethoxyphenyl)alanine *L*-phenylethylamine salt, dissolving the crystalline salt in dilute aqueous alkali, extracting the resulting solution with a water-immiscible organic solvent to remove *L*- α -phenylethylamine, acidifying the aqueous solution with a dilute aqueous mineral acid to form *L*-*N*-acetyl- α -methyl- β -(3,4-dimethoxyphenyl)alanine, and hydrolysing the latter with a solution of aqueous hydrobromic acid having a concentration of about 30% to about 50% by weight at a temperature in the range of about 90°C. to reflux temperature to form *L*- α -methyl- β -(3,4-dihydroxyphenyl)alanine.

This compound can also be prepared from its racemic form by heating the latter with an excess of acetic anhydride in the presence of a proton acceptor to form *DL*-*N*-acetyl- α -methyl- β -(3,4-diacetoxyphenyl)alanine, agitating the *DL*-triacyl alanine with quinine free base in acetone, cooling the reac-

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tion mixture to precipitate the *L*-triacetyl alanine quinine salt, recovering the latter from the mixture and heating it with aqueous acid, or by heating the racemic form of the alanine with an excess of acetic anhydride in the presence of a proton acceptor to form *DL*-*N*-acetyl- α -methyl- β -(3,4-diacetoxyphenyl)alanine, heating the *DL*-triacetyl alanine with quinine free base in acetone, recovering from the cooled solution the *L*-triacetyl alanine quinine salt, adding a solution of this salt in methanol to an aqueous solution of sodium oxalate, separating the diquinine oxalate, removing solvents by evaporation, and removing the acetyl groups by hydrolysis.

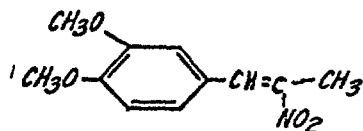
In this use *in vivo* the optically active compounds of the invention may be given orally or parenterally. Dosage for oral administration will vary from 10–500 mg. per kg. per day. For oral administration to humans, the dosage range is from 0.1 to 5.0 g. per day, preferably 0.5 to 1.5 g. per day, usually in frequent small doses. The esters, both the carboxy type and the acyloxy type, because of their better absorption by the organisms, have a lower preferred range of the order of 0.2 to 2.5 g. per day, and may be given in less frequent larger doses.

These compounds of the invention can be mixed with any of the conventional ingredients for tablets. They may also be given parenterally and can be mixed with any of the conventional materials and carriers used in composition for that method of application. They may also be mixed with any other anti-hypertensive agents in combination.

The invention is illustrated by the following examples, certain of which show the preparation of suitable intermediates. The words 'Amberlite', 'Super-cel' and 'Darco' used in the examples are trade marks. All specific rotations were determined at 25°C.

A. Preparation Of Starting Ketones

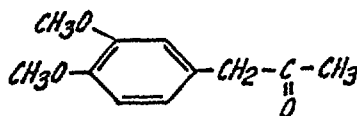
EXAMPLE 1



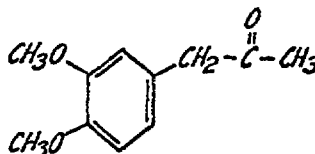
To a solution of 62.9 g. of 3,4-dimethoxybenzaldehyde in 121 ml. of toluene is added 50.1 g. of nitroethane, 3.03 ml. of *n*-butylamine and 3.60 ml. of glacial acetic acid. The mixture is refluxed and the water of reaction is steadily azeotropically removed by distillation. After the theoretical amount of water has been distilled out, distillation is continued to remove excess of reactants. The last trace

of any excess of reactants is then removed at room temperature under vacuum. The product is then triturated with a hydrocarbon solvent such as Skellysolve B and is thus obtained in a crystalline state. In general, however, it is preferred to dissolve the residue directly in toluene for use in the next step (see Example 2), without isolating the 1-(2-nitroprop-1-yl)-3,4-dimethoxybenzene.

EXAMPLE 2

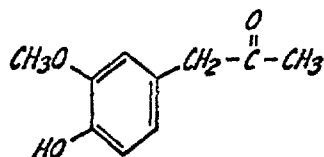


To a mixture of 137.4 g. of 40-mesh iron, 2.75 g. of hydrated ferric chloride and 172 ml. of water, there is added 84.6 g. of 1-(2-nitroprop-1-yl)-3,4-dimethoxybenzene (the product of Example 1) dissolved in 50 ml. of toluene. The mixture is heated to reflux and 248 ml. of concentrated hydrochloric acid is added dropwise at a rate calculated to keep the mixture refluxing vigorously. After addition of all the hydrochloric acid, the refluxing is continued by the application of heat for several hours. A silicaceous filter aid is then added to the cooled reaction mixture and the material is removed by filtration. The filter cake is washed four times, each time with 90 ml. of benzene. The organic layer is then separated from the filtrate. The water layer is acidified to a pH of 2 and extracted three times with 90-ml. portions of benzene. These extracts are then combined with the organic solvent layer and the combined organic phase is extracted four times with 100-ml. portions of water. It is then stirred for an hour with 230 ml. of 10% sodium bisulphite solution. The organic solvent phase is then separated, washed seven times with 100-ml. portions of water and dried over magnesium sulphate. Evaporation of the solvent gives 1-(3,4-dimethoxyphenyl)-2-propanone in the form of an oil.



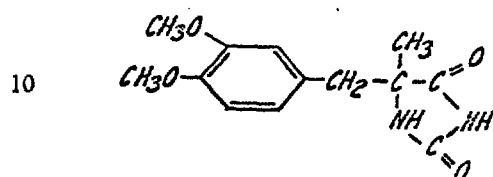
1-(3,4-dimethoxyphenyl)-2-propanone, can be prepared by the procedures of Examples 17, 18 and 19 of U.S. Patent 2,868,818, where it is named methyl-(3,4-dimethoxybenzyl)-ketone.

EXAMPLE 3



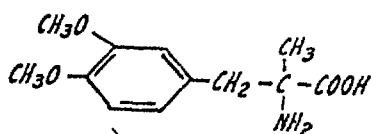
The procedures of Examples 1 and 2 are followed using an equivalent quantity of vanillin in place of the dimethoxybenzaldehyde used in Example 1 to give 1-(4-hydroxy-3-methoxyphenyl)-2-propanone.

B. Synthesis Of Racemic Amino Acids
EXAMPLE 4



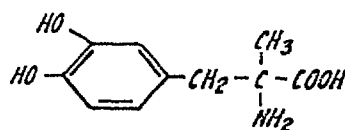
To a solution of 165 g. of ammonium carbonate and 35.4 g. of potassium cyanide in 484 ml. of water, there is added 41.5 g. of 1-(3,4-dimethoxyphenyl)-2-propanone (the product of Example 2) in 484 ml. of ethanol while keeping the temperature at 25–60°C. The mixture is stirred and held at 55–60°C. for 18 hours. It is then concentrated to one-third of its original volume under a vacuum. The mixture is then chilled in an ice bath and the crystalline 5-methyl-5-(3,4-dimethoxybenzyl)hydantoin is filtered, washed and dried. It may be recrystallized from 50% aqueous ethanol.

EXAMPLE 5



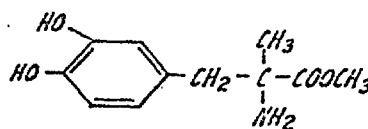
An autoclave is charged with 9 g. of the product of Example 4, 45 g. of barium hydroxide octahydrate and 226 ml. of water. The autoclave is then heated at 150° for 18 hours. The charge is then removed and carbon dioxide is passed through the mixture at 50°C. The precipitated barium carbonate is removed by filtration and exhaustively washed with hot water. The filtrate and washings, having a pH of 7.8, are treated with 2N sulphuric acid until the pH is 6.4. The mixture is then filtered while hot through a siliceous filter aid and the cake is washed with hot water. The filtrate is concentrated to dryness to yield the desired α -methyl-3,4-dimethoxyphenylalanine.

EXAMPLE 6



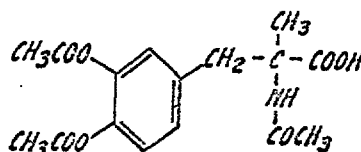
To 124 ml. of 48% hydrobromic acid there is added 8.15 g. of α -methyl- β -(3,4-dimethoxyphenyl)-alanine (the product of Example 5). The mixture is purged with nitrogen and then refluxed for 5 hours. It is then concentrated to near dryness under a vacuum. The resulting gum is then flushed twice, alternately, with each of tertiary butanol and water. The hydrobromide salt of the product is dissolved in water and passed through a column containing 41.5 g. of weakly basic ion exchange resin (Amberlite IR-45) on the hydroxide cycle (1.85 mg/g.). The column is eluted with 800 ml. of water. The eluate is concentrated under reduced pressure to 20 ml. at which point the α -methyl- β -(3,4-dihydroxyphenyl)alanine crystallizes out. The product is filtered and washed with isopropyl alcohol.

EXAMPLE 7



A suspension of 23 g. of the product of Example 6 in 250 ml. of anhydrous methanol is saturated with hydrogen chloride while being held at 10–20°C. The mixture is then refluxed for three hours after which it is allowed to stand for 18 hours. The solvent is removed under a vacuum and the residue is redissolved in 50 ml. of methanol under nitrogen. The methanol is then removed under a vacuum. The addition of methanol is twice repeated. The resulting hydrochloride salt of the methyl ester is then taken up in 50 ml. of water at 50°C. The solution is filtered and the pH of the filtrate is adjusted to 8.5 with concentrated ammonium hydroxide keeping the mixture under nitrogen at 5–10°C. The mixture is then allowed to stand for 6 hours at 5°, under nitrogen, after which it is filtered and the isolated product is washed three times with 15 ml. of water at 0° and then dried in a vacuum.

EXAMPLE 8

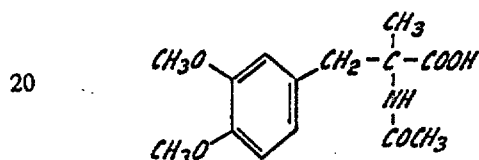


A mixture of 25 g. of DL- α -methyl-

β - 3,4 - dihydroxyphenyl alanine, 100 ml. of acetic anhydride and 75 ml. of pyridine is purged thoroughly with nitrogen. It is then heated to 90°C. for 2 hours. The mixture is allowed to stand overnight at room temperature, after which it is concentrated *in vacuo* on a steam bath to a thick syrup. The residue is stirred with ice water and made strongly acidic with 2.5 N hydrochloric acid. The precipitated crystalline *DL* - triacetyl compound is isolated by filtration, washed thoroughly with ice water and dried over P_2O_5 *in vacuo*.

When the acetic anhydride in the above process is replaced by an equivalent quantity of propionic anhydride, butyric anhydride or formic acetic anhydride, the corresponding propionyl, butyryl and formyl derivatives are obtained.

EXAMPLE 9



A mixture of 69 g. of *DL* - α - methyl - β - (3,4 - dimethoxyphenyl) alanine (prepared as an intermediate in Example 5), 276 ml. of acetic anhydride and 207 ml. of pyridine are mixed and heated with stirring on a steam bath for three hours. The reaction mixture is allowed to cool and to stand at room temperature for an additional 18 hours. The mixture is then concentrated to a thick syrup. The syrup is poured with stirring into a beaker containing 300 ml. of ice water. The mixture is stirred for ten minutes and then made strongly acid with 150 ml. of a 2.5N hydrochloric acid. The resulting mixture, which is very thick, is cooled to 5°C., and the product, *DL* - *N* - acetyl - α - methyl - β - (3,4 - dimethoxyphenyl)alanine, filtered off, washed with cold water and then with 100 ml. of absolute ethanol, and dried over phosphorus pentoxide *in vacuo*.

EXAMPLE 10

α - methyl - β - (3,4 - dihydroxyphenyl) - alanine.

A mixture of 23.56 g. of methyl - (3,4 - dimethoxy - benzyl) - ketone, a solution of 8.2 g. of potassium cyanide in 14 ml. water, and a solution of 6.6 g. of ammonium chloride in about 60 ml. water, was heated and stirred at 55° to 60°C. for 5.5 hours and then at room temperature overnight. The yellow lower oily layer became brown in colour during the heating period. The hydrogen chloride acid-addition salt of 1 - (3,4 - dimethoxyphenyl) - 2 - amino - 2 - cyano - propane, which precipitated as a crystalline solid, was separated from the

liquid by filtration, washed with water, and dried at room temperature. The yield was 20 g. (77%) of the aminonitrile, m.p. 103—106°C. with decomposition. To 19 g. of this nitrile was added 90 ml. of concentrated hydrochloric acid and the slightly turbid solution was saturated with hydrogen chloride at room temperature. This treatment resulted in conversion of the nitrile group ($-\text{CN}$) to an acid amide group ($-\text{CONH}_2$). The reaction mixture was then heated on the steam bath for 18 hours to hydrolyse the acid amide group to the free acid group ($-\text{COOH}$) and the 3-methoxy substituent to 3-hydroxy. The solution was cooled and filtered to remove a small amount of precipitated ammonium chloride. The filtrate was concentrated to dryness *in vacuo* on the steam bath, the residue was dissolved in 20 ml. hot water, and the pH of the solution adjusted to 6.5 with concentrated ammonium hydroxide. Upon cooling the solution the product, *DL* - α - methyl - β - (3 - hydroxy - 4 - methoxyphenyl)alanine, crystallized out and was filtered, washed with alcohol and ether and dried. The compound was purified by dissolving in boiling water, treating with activated carbon, and concentrating the clear filtrate to the point of crystallization. M.P. 295—296°C. with decomposition. One gram of the α - methyl - β - (3 - hydroxy - 4 - methoxyphenyl) - alanine was dissolved in 40 ml. of concentrated hydrochloric acid, the solution saturated with hydrogen chloride and heated in a sealed tube at 150°C. for 2 hours. The dark reaction mixture was concentrated to dryness *in vacuo* and excess acid was removed by flushing several times with ethanol. On dissolving the dark residue in a minimum amount of water and adjusting the clarified solution to pH 6.5 with ammonium hydroxide the *DL* - α - methyl - β - (3,4 - dihydroxyphenyl)alanine separated in fine crystals which were filtered and washed with alcohol and ether. The crystalline product had a melting point of 299.5—300°C. with decomposition.

C. Resolution Of Amino Acids

EXAMPLE 11

L - *N* - acetyl - α - methyl - β - (3,4 - dimethoxyphenyl)alanine - *L* - α - phenylethylamine salt.

To a slurry of 2.1 g. of *DL* - *N* - acetyl - α - methyl - β - (3,4 - dimethoxyphenyl) - alanine in 4 ml. of methanol is added a solution of 0.91 g. of *L* - α - phenylethylamine in 1 ml. of methanol. The mixture is heated to reflux, diluted with 10 ml. of methanol and refluxed until solution is complete. The solution is clarified by filtration through a filter containing a diatomaceous earth ("Super-Cel"). The filter cake is washed with 10 ml. of water. The filtrate is heated allowing the

methanol to distil until the internal temperature is 95°C. The solution is cooled to 60°C., seeded with the product, *L* - *N* - acetyl - α - methyl - β - (3,4 - dimethoxyphenyl)alanine *L* - α - phenylethylamine salt, and allowed to cool undisturbed to room temperature. The mixture is then aged at 8°C. in a refrigerator for an additional 18 hours. At the end of this aging period approximately half of the product, *L* - *N* - acetyl - α - methyl - β - (3,4 - dimethoxyphenyl)alanine - *L* - α - phenylethylamine salt, has crystallized in the form of striated mounds. Seed crystals are formed by swirling the flask occasionally. The mixture is then allowed to stand for an additional 24 hours. The crude product *L* - *N* - acetyl - α - methyl - β - (3,4 - dimethoxyphenyl)alanine *L* - α - phenylethylamine salt is filtered, washed with cold water and dried *in vacuo* at 56°C. The specific rotation is $[\alpha]_D^{25} +55^\circ$ (*C* = 1 in methanol). The mother liquors are collected for recovery of impure *D* - *N* - acetyl - α - methyl - β - (3,4 - dimethoxyphenyl)alanine.

To 1.38 g. of the crude product are added 10 ml. of water and 2 ml. of ethanol. The mixture is heated without a condenser with stirring until complete solution occurs and the internal temperature is 98°C. The solution is allowed to cool slowly to room temperature and then aged at 8°C. for two hours. The semi-solid mixture is filtered, washed with a small amount of cold water and dried at 56°C. *in vacuo*, yielding pure *L* - *N* - acetyl - α - methyl - β - (3,4 - dimethoxyphenyl)alanine *L* - α - phenylethylamine salt. $[\alpha]_D^{25} +69^\circ$. (*C* = 1 in methanol).

EXAMPLE 12

D - *N* - acetyl - α - methyl - β - (3,4 - dimethoxyphenyl)alanine - *D* - α - phenylethylamine salt and the corresponding *L* - *L* salt.

To a slurry of 77 g. of *DL* - *N* - acetyl - α - methyl - β - (3,4 - dimethoxyphenyl)alanine in 200 ml. of methanol is added 33.2 g. of *D* - (+) - α - phenylethylamine in 50 ml. of methanol. One litre of water is added and the methanol stripped *in vacuo* at 50° to 60°C. The aqueous solution is heated to 90° and clarified by filtration through diatomaceous earth ("Super-Cel"). The filtrate is seeded with *D* - *N* - acetyl - α - methyl - β - (3,4 - dimethoxyphenyl)alanine *D* - α - phenylethylamine salt and allowed to cool undisturbed to room temperature. The mixture is aged at about 8°C. for 18 hours and filtered, and the precipitate of *D* - *N* - acetyl - α - methyl - β - (3,4 - dimethoxyphenyl)alanine *D* - α - phenylethylamine salt is washed with a small amount of cold water and dried over phosphorus pentoxide *in vacuo*. $[\alpha]_D^{25} -59^\circ$ (*C* = 1 in methanol). This salt has a negative rotation and the product of Example 10 has a positive rotation. A second crop is obtained by con-

centration of the mother liquors to a volume of about 700 ml. and aging at 5°—10°C. for 18 hours. A third crop is obtained by further concentration of the mother liquors to 400 ml. and aging at room temperature for 2 hours.

The remaining mother liquors are made alkaline with 50 ml. of 2.5*N* sodium hydroxide and extracted with chloroform in two 100 ml. portions followed by two 50-ml. portions. To the basic aqueous solution is added 15 ml. of glacial acetic acid, and the solution is aged for 18 hours at about 8°C. The precipitate of impure *L* - *N* - acetyl - α - methyl - β - (3,4 - dimethoxyphenyl)alanine is filtered, washed with cold water and dried *in vacuo* at 70°C. $[\alpha]_D^{25} -23^\circ$ (*C* = 1 in methanol).

To a slurry of 30 g. of the *L* - *N* - acetyl - α - methyl - β - (3,4 - dimethoxyphenyl)alanine obtained above in 200 ml. of methanol is added 13.0 g. of *L* - (-) - α - phenylethylamine. A thick precipitate forms. This precipitate is dissolved by addition of 200 g. more methanol and 100 ml. of water and heating to reflux. The hot solution is treated with decolorizing charcoal, filtered and allowed to cool to room temperature. The mixture is aged at 10°C. for one hour and filtered. The precipitate of *L* - *N* - acetyl - α - methyl - β - (3,4 - dimethoxyphenyl)alanine *L* - (-) - α - phenylethylamine salt is washed with cold water and dried over phosphorus pentoxide for about 18 hours. $[\alpha]_D^{25} +68^\circ$ (*C* = 1 in methanol).

EXAMPLE 13

L - *N* - acetyl - α - methyl - β - (3,4 - dimethoxyphenyl)alanine

25 g. of *L* - *N* - acetyl - α - methyl - β - (3,4 - dimethoxyphenyl)alanine *L* - α - phenylethylamine salt (the product of Examples 11 and 12) is dissolved in 100 ml. of water and 27.5 ml. of 2.5*N* sodium hydroxide. The solution is extracted with two 50-ml. portions followed by two 25-ml. portions of chloroform, and the extracts are saved for the recovery of *L* - α - phenylethylamine. The extracted aqueous solution is partially acidified with 6 ml. of 2.5*N* hydrochloric acid, heated to 70°C., transferred to a beaker and further acidified with 24 ml. of 2.5*N* hydrochloric acid. A precipitate of *L* - *N* - acetyl - α - methyl - β - (3,4 - dimethoxyphenyl)alanine forms immediately. The precipitate is cooled to 10°C., filtered, washed with cold water and dried *in vacuo* at 70°C. $[\alpha]_D^{25} -56^\circ$ (*C* = 1 in methanol).

EXAMPLE 14

L - α - methyl - β - (3,4 - dihydroxyphenyl)alanine

A mixture of 10.0 g. of *L* - *N* - acetyl - α - methyl - β - (3,4 - dimethoxyphenyl)alanine and 100 ml. of 48% by weight aqueous hydrobromic acid is purged with nitrogen and heated at reflux in a nitrogen atmosphere for 3—12 hours. The system is protected from air by a mercury trap. The mixture is con-

concentrated to dryness under nitrogen. The residue is dissolved in 50 ml. of water and concentrated to dryness. This procedure is repeated successively with 50 ml. of *t*-butanol and 50 ml. of water. The residue is dissolved in 80 ml. of water, the pH adjusted to 6.4 with 6*N* ammonium hydroxide and the mixture is treated with 0.2 g. of decolorizing charcoal ("Darco G-60") and filtered while warm. The amber-coloured solution is cooled, saturated with sulphur dioxide and concentrated under nitrogen to a volume of 30 ml. The mixture is aged in an ice bath for one hour and filtered. The cake is washed with a minimum amount of cold water and dried at 56°C. *in vacuo*. The resulting product is *L* - α - methyl - β - (3,4 - dihydroxyphenyl)alanine (V). $[\alpha]_D^{25} -4.0 \pm 2.0$ ($C=1$ in 1*N* hydrochloric acid).

A second crop of crystals weighing 0.6 g. and having a negative bromide test is obtained by aging the mother liquors for 3 days at 8°C.

EXAMPLE 15

Ethyl ester of *L* - α - methyl - β - (3,4 - dihydroxyphenyl)alanine

A suspension of 95 g. (0.45 mole) of *L* - α - methyl - β - (3,4 - dihydroxyphenyl)alanine in 950 ml. of absolute ethanol is saturated with dry hydrogen chloride at 10–20°C. and then refluxed for two hours. The reaction mixture is concentrated *in vacuo*. The residue is dissolved in absolute ethanol, and the solution is again concentrated *in vacuo*. This residue is dissolved in absolute ethanol, and the solution is saturated with dry hydrogen chloride at 10–20°C. and refluxed for two - and - a - half hours. The reaction mixture is concentrated *in vacuo*. The residue is dissolved in absolute ethanol followed by concentration. The procedure is repeated an additional two times. The residue from the final concentration is allowed to stand until crystals of the ethyl ester hydrochloride of α - methyl - β - (3,4 - dihydroxyphenyl)alanine formed. A sample is allowed to stand in air and form a hydrate.

To the ethyl ester hydrochloride and mother liquors is added 420 ml. of 1.059*N* sodium ethoxide, corresponding to about one mole per mole of ester hydrochloride. This results in an ethanol solution of the ethyl ester. This solution is concentrated to dryness. The residue is partitioned between methylene chloride and water, and the aqueous layer is discarded. The methylene chloride layer is washed with water, dried over anhydrous sodium sulphate, and concentrated *in vacuo* until all methylene chloride is removed. The alkyl ester of *L* - α - methyl - β - (3,4 - dihydroxyphenyl)alanine is obtained, as an amorphous material. $[\alpha]_D^{25} -10.0$ ($C=1$ in 1*N* HCl).

When methanol, isopropanol, lauryl alcohol or ~~octadecyl~~ alcohol is used in place of the ethanol (the higher alcohols must be heated to

render them liquid) in the above procedure, the corresponding methyl, *i*-propyl, lauryl and ~~octadecyl~~ esters are obtained.

EXAMPLE 16

L - *N* - acetyl - α - methyl - β - (3,4 - diacetoxyphenyl)alanine quinine salt

Step A

A mixture of 96.4 g. of quinine free base and 100 g. of *DL* - *N* - acetyl - α - methyl - β - (3,4 - diacetoxyphenyl)alanine is charged to a flask and 960 ml. of acetone is added. The mixture is stirred until solution is completed and is then cooled to 0–5° for 4 hours. The desired precipitated salt is isolated by filtration, washed three times with 100 ml. batches of acetone and dried under a vacuum at 40°C. $[\alpha]_D^{25} -72.5^\circ$ ($C=1$ -96% ethanol).

L - *N* - acetyl - α - methyl - β - (3,4 - diacetoxyphenyl)alanine

Step B

The *L*-quinine salt obtained from Step A is treated with a cold solution (0.5°) of 11.0 ml. of 2.5*M* hydrochloric acid in 60 ml. of water. To the clear solution is added 10.6 ml. of 2.5*M* hydrochloric acid, which causes the product to precipitate. The mixture is held overnight at 0–5°, after which the product is filtered off, washed with water and dried under a vacuum. $[\alpha]_D^{25} -71.7^\circ$.

L - α - Methyl - β - (3,4 - dihydroxyphenyl)alanine

Step C

A solution of *L* - *N* - acetyl - α - methyl - β - (3,4 - diacetoxyphenyl)alanine (the product of Step B) (25.0 g.) in 200 ml. of 6*M* hydrochloric acid is refluxed two hours. The solvent is removed *in vacuo* and the residual yellow oil is flushed three times with 50 ml. portions of *tert*-butyl alcohol until the hydrochloric acid is completely removed. The gummy residue is dissolved in 45 ml. of water and filtered to remove trace amounts of insolubles. The aqueous solution is treated with 7.5 ml. of concentrated ammonia to bring the pH to 7.5 and then with 1.0 g. of sulphur dioxide. The desired product separates and the mixture is held at 0–5° overnight. The crystals are isolated by filtration, washed twice with 10 ml. portions of cold water and dried under a vacuum. $[\alpha]_D^{25} -4.0 \pm 2.0^\circ$.

EXAMPLE 17

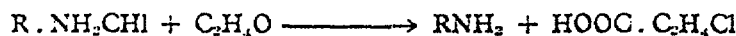
L - α - methyl - β - (3,4 - dihydroxyphenyl)alanine

An alternative procedure for conversion of crystalline quinine salt to the free acid is as follows:

The viscous solution obtained by dissolving 100 g. of *L* - *N* - acetyl - α - methyl - β - (3,4 - diacetoxyphenyl)alanine quinine salt (the product of Example 16 Step A) in 100 ml. of methanol is added at 65°C. with stirring to a solution of 10.2 g. of sodium oxalate in 500

octadecyl

ml. of water. The transfer is completed with 15 ml. of methanol. The heavy slurry of diquinine oxalate is cooled at 0—5° for 2 hours, filtered, washed three times with 80 ml. of water, and dried under a vacuum at 50°. The water-clear, neutral mother liquors and washes were distilled at atmospheric pressure to remove methanol. When the distillation temperature reaches 100°, distillation is stopped, 500 ml. of concentrated hydrochloric acid is added and the solution is heated at

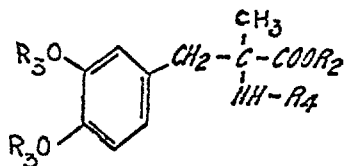


The product is filtered, washed with acetone and dried under a vacuum at 50°C. The crude product is contaminated with chloride ion, slurried in 350 ml. of water, and the pH is adjusted to 6.5 with 0.5 ml. of concentrated ammonium hydroxide and treated with 7.5 g. of sulphur dioxide. The mixture is stirred for 15 minutes and the insolubles are removed by filtration, washed with water and dried. The product thus obtained is *L* - α - methyl - β - (3,4 - dihydroxyphenyl)alanine. $[\alpha]_D -1.50$ (1*N* HCl).

Further crops of the product can be obtained by treatment of the filtrate with activated charcoal and concentration.

WHAT WE CLAIM IS:—

1. Compounds of the formula:—



in which, R_2 is a hydrogen atom or an alkyl radical and each of R_1 and R_4 is a hydrogen atom or C_{1-6} alkanoyl radical, the said compound being in the *L* (sinister) absolute spatial configuration at the α -carbon atom and substantially free of the *D* (rectus) absolute configuration enantiomorph.

2. *L* - α - methyl - β - (3,4 - dihydroxyphenyl)alanine substantially free of its *D* enantiomorph.

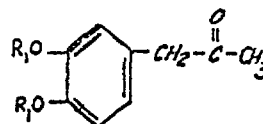
3. *L* - α - methyl - β - (3,4 - dihydroxyphenyl)alanine ethyl ester substantially free of its *D* enantiomorph.

4. *L* - *N* - acetyl - α - methyl - β - (3,4 - diacetoxyphenyl)alanine substantially free of its *D* enantiomorph.

5. A non-toxic salt of a compound as claimed in any preceding claim.

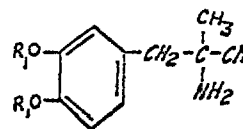
6. A process for preparing the compound claimed in Claim 2, which comprises heating a compound of the formula:—

reflux for two hours. The reaction mixture is then concentrated to dryness under vacuum, flushed with three 100-ml. portions of tert-butyl alcohol, and the residue is dissolved in 100 ml. of acetone. Sodium chloride is removed by filtration and the acetone filtrate treated with 13.8 g. of ethylene oxide and allowed to stand at 10°C. for several days. The effect of the ethylene oxide is to liberate the amine without going past the isoelectric point



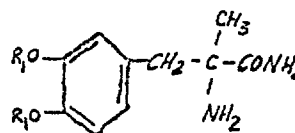
(Formula I),

in which each of R_1 is a hydrogen atom or a methyl group, at least one being methyl with ammonium halide and a water-soluble cyanide salt in aqueous solution to form a compound of the formula:



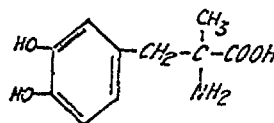
(Formula II),

treating the latter with an aqueous acid to form a compound of the formula:—



(Formula IV),

heating this with an aqueous hydrohalic acid under pressure to produce a compound of the formula:



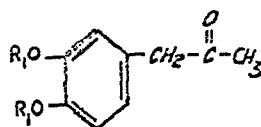
(Formula V),

and either (a) resolving the compound of formula V into its optically active components by reaction with an optically active base so as to form a salt with the carboxy group or (b) effecting the resolution into optically active components of the compound of Formula II or any compound formed at a subsequent stage

in the synthesis by microbiological means or by the action of an optically active acid on the α -amino group, the steps after the resolution being effected on the isomer having the isolated *L* (sinister) absolute spatial configuration.

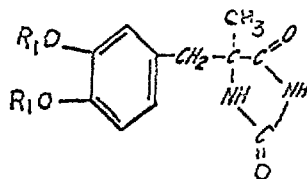
5 7. A process as claimed in Claim 6, in which the ammonium halide used in the first step is ammonium chloride.

10 8. A process for preparing the compound as claimed in Claim 2, which comprises reacting a compound of the formula:



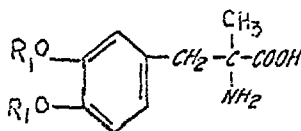
(Formula I),

15 in which R_1 is as defined in Claim 6, with ammonium carbonate and a water-soluble cyanide salt in aqueous solution to form a compound of the formula:—



(Formula III),

20 heating the compound of Formula III with an aqueous concentrated hydrohalic acid to produce a compound having the Formula V set forth in Claim 6, or heating the compound of Formula III with an aqueous base to produce a compound of formula



(Formula VI)

25 demethylating the compound of Formula VI to produce a compound having the Formula V set forth in Claim 6 and either (a) resolving the compound of Formula VI into its optically active compounds by reaction with an optically active base so as to form a salt with the carboxy group or (b) effecting the resolution into optically active components of the compounds of Formula III or any compound
30 formed at a subsequent stage in the synthesis by microbiological means or by the action of an optically active acid on the α -amino group, the steps after the resolution being effected on the isomer having the isolated *L* (sinister)
35 absolute spatial configuration.

40 9. A process as claimed in Claim 6, 7 or 8,

including one or both (in either order) of the following additional steps, viz.,

(a) heating with an alkanol in the presence of a mineral acid to convert the carboxy group to a carboalkoxy group, with the proviso that this step must be carried out after the resolution if it is carried out by method (a) of Claims 6 and 8;

(b) heating with a C_{1-4} alkanolic anhydride in the presence of a proton acceptor to acylate the amino group and any nuclear hydroxyl groups; the demethylation step of Claim 8 being carried out before or after the or either or both of the additional steps.

10. A process for preparing *L* - α - methyl - β - (3,4 - dihydroxyphenyl)alanine substantially free of its *D*-enantiomorph which comprises reacting *DL* - α - methyl - β - (3,4 - dimethoxyphenyl)alanine with acetic anhydride in the presence of a proton acceptor, thereby forming *DL* - *N* - acetyl - α - methyl - β - (3,4 - dimethoxyphenyl)alanine, reacting this compound with an optically active *L* - α - phenylethylamine in an organic solvent at elevated temperature, recovering crystalline *L* - *N* - acetyl - α - methyl - β - (3,4 - dimethoxyphenyl)alanine *L* - phenylethylamine salt, dissolving the crystalline salt in dilute aqueous alkali, extracting the resulting solution with a water-immiscible organic solvent to remove *L* - α - phenylethylamine, acidifying the aqueous solution with a dilute aqueous mineral acid to form *L* - *N* - acetyl - α - methyl - β - (3,4 - dimethoxyphenyl)alanine, and hydrolysing the latter with a solution of aqueous hydrobromic acid having a concentration of about 30% to about 50% by weight at a temperature in the range of about 90°C. to reflux temperature to form *L* - α - methyl - β - (3,4 - dihydroxyphenyl)alanine.

11. A process for obtaining *L* - α - methyl - β - (3,4 - dihydroxyphenyl)alanine substantially free of its *D* enantiomorph which comprises heating the racemic form of the alanine with an excess of acetic anhydride in the presence of a proton acceptor to form *DL* - *N* - acetyl - α - methyl - β - (3,4 - diacetoxyphenyl)alanine, agitating the *DL*-tri-acetyl alanine with quinine free base in acetone, cooling the reaction mixture to precipitate the *L*-triacetyl alanine quinine salt, recovering the latter from the mixture and heating it with aqueous acid and recovering from the reaction mixture *L* - α - methyl - β - (3,4 - dihydroxyphenyl)alanine substantially free of its *D* enantiomorph.

12. A process for obtaining *L* - α - methyl - β - (3,4 - dihydroxyphenyl)alanine substantially free of its *D* enantiomorph which comprises heating the racemic form of the alanine with an excess of acetic anhydride in the presence of a proton acceptor to form *DL* -



- N - acetyl - α - methyl - β - (3,4 - diacetoxyphenyl)alanine, heating the *DL*-triacetyl alanine with quinine free base in acetone, recovering from the cooled solution the *L*-triacetyl alanine quinine salt, adding a solution of this salt in methanol to an aqueous solution of sodium oxalate, separating the diquinine oxalate, removing solvents by evaporation, removing the acetyl groups by hydrolysis and recovering the *L* - α - methyl - β - (3,4 - dihydroxyphenyl)alanine.
13. A process for preparing a compound as claimed in Claim 1, substantially as hereinbefore described with reference to any of the appropriate foregoing Examples.
14. A process for preparing a compound as claimed in Claim 1, substantially as hereinbefore described with reference to either of reaction schemes 2 or 3 above.
15. A compound as claimed in Claim 1, when prepared by a process as claimed in any one of Claims 6—14 or its obvious chemical equivalent.
16. A pharmaceutical composition comprising a compound as claimed in any one of Claims 1—5 and 15 in which R_1 and R_2 are hydrogen atoms, together with an inert pharmaceutical diluent.
17. A pharmaceutical composition as claimed in Claim 16, in the form of a tablet.
18. A pharmaceutical composition as claimed in Claim 16, in a form suitable for parenteral administration.
- For the Applicants, 
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