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L- α -HYDRAZINO - α -SUBSTITUTED - β - (3,4 - DIHYDROXYPHENYL)PROPIONIC ACID SUBSTANTIALLY FREE OF THE D ISOMER, THE LOWER ALKYL ESTERS THEREOF, AND THE PHARMACEUTICALLY ACCEPTABLE SALTS THEREOF, WHEREIN THE SUBSTITUENT IS LOWER ALKYL

Sandor Karady, Mountainside, Manuel G. Ly, Edison, Seemon H. Pines, Murray Hill, and Meyer Sletzing, North Plainfield, N.J., assignors to Merck & Co., Inc., Rahway, N.J.

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6 Claims

ABSTRACT OF THE DISCLOSURE

A novel compound is used to inhibit mammalian decarboxylase, the compound being selected from the group consisting of L- α -hydrazino- α -substituted - β - (3,4-dihydroxyphenyl)propionic acid substantially free of the D isomer, the lower alkyl esters thereof, and the pharmaceutically acceptable salts thereof, wherein the substituent is lower alkyl. Compounds, compositions and method of treatment are included.

The present invention relates to novel and useful compounds. More particularly, it relates to novel compounds which are particularly useful for inhibiting decarboxylase in mammals.

It is known in the art that racemates of α -hydrazino- α -substituted- β -(3,4-dihydroxyphenyl)propionic acids and its esters are very potent decarboxylase inhibitors in mammals. See Sletzing et al. "Journal of Medicinal Chemistry," Vol. 6, page 101 (1963) and Porter et al. "Biochemical Pharmacology," Vol. 11, page 1067 (November 1962). Such compounds have found widespread use in the pharmaceutical field. It has now been found that the D isomer of the racemate is essentially inactive and is even antagonistic to the action of the L form thereby reducing its potency. Thus, in some tests it appears that the L form of the compound is the only active form and the D form is completely inactive. In other tests it appears that the D form will actually detract from the action of the L form. In any event, the present invention provides a much more potent decarboxylase inhibitor than was heretofore available to the medical profession.

The inhibition of mammalian decarboxylase is an important part of the physiological action of many types of drugs. For example, it has recently been proposed to use L-dopa in the treatment of Parkinson's disease. However, L-dopa is utilized both in the brain and the peripheral parts of the body and it is desired that it only be utilized in the brain. The present hydrazine compounds do not pass the blood brain barrier and hence inhibit decarboxylase only in the peripheral parts of the body. Thus, when L-dopa is used in conjunction with the hydrazine compounds of the present invention, the decarboxylase of L-dopa is inhibited only in the peripheral parts of the body making more of it available to the brain. The net

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result is that much less L-dopa is required for effective medication.

The inhibition of decarboxylase is also of importance in the treatment of certain disorders of the colon. In some persons, the cells in the intestines, and perhaps elsewhere, develop over activity in the production of serotonin from 5-hydroxytryptophane. The result of such over abundance of serotonin is constant flushing of the colon and evacuation of the bowels. Further, unless this condition is controlled, it can develop into much more serious trouble. Decarboxylase inhibitors prevent the formation of the serotonin and therefore control such diarrhea. Powerful decarboxylase inhibitors such as the hydrazino compounds used in this invention, especially those having no other physiological activity, are peculiarly adapted to such use.

The compounds used in our invention inhibit not only dioxyphenylalanine decarboxylase but also histidine decarboxylase. They thus show promise of use as antihistaminics as well.

It is an object of the present invention to provide a new and useful decarboxylase inhibitor. A further object is to provide a much more potent decarboxylase inhibitor. Another object is to provide a method for inhibiting decarboxylase in mammals. A still further object is to provide a composition which is suitable for inhibiting decarboxylase in mammals. Other objects will become apparent as the description of the invention proceeds.

These objects are accomplished by the present invention which provides a novel compound selected from the group consisting of (A) L- α -hydrazino- α -substituted- β -(3,4-dihydroxyphenyl)propionic acid substantially free of the D isomer, (B) the lower alkyl esters thereof and (C) the pharmaceutically acceptable salts thereof, wherein the substituent is lower alkyl.

In a more preferred embodiment of the present invention the substituent is methyl or ethyl and the compounds are used as the free base.

The present invention also provides a method of inhibiting mammalian decarboxylase which comprises administering to the mammal from 0.05 to 100 mg./kg. per day of a compound selected from the group consisting of (A) L- α -hydrazino - α - substituted - β - (3,4-dihydroxyphenyl)-propionic acid substantially free of the D isomer, (B) the lower alkyl esters thereof and (C) the pharmaceutically acceptable salts thereof, wherein the substituent is lower alkyl.

The present invention further provides a pharmaceutical composition comprising an inert pharmaceutical carrier and from about 5 mg. to about 15 g. of a compound selected from the group consisting of (A) L- α -hydrazino- α -substituted- β -(3,4-dihydroxyphenyl)propionic acid substantially free of the D isomer, (B) the lower alkyl esters thereof and (C) the pharmaceutically acceptable salts thereof, wherein the substituent is lower alkyl.

The "pharmaceutically acceptable salts" of the compounds which may be used include, without limitation, the alkali metal and ammonium salts of the carboxy function and the hydrochloride, hydrobromide, sulfate and the like salts of the amino function. In a preferred embodiment of the invention, the free base compounds are used and not the salts. The terminology "substantially free of the D isomer" signifies that less than 10% of D isomer is present. The "lower alkyl" substituent or ester group may contain from 1 to about 5 carbon atoms, but preferably contains 1, 2 or 3 carbon atoms.

The following examples are given to illustrate the invention and are not intended to limit it in any manner. All parts are given in parts by weight unless otherwise expressed.

EXAMPLE 1

A. Preparation of L-4-(3,4-dimethoxybenzyl)-4-methyl-hydantoic acid

To a solution of L- α -methyl-(3,4-dihydroxyphenyl) alanine (100 g., 0.47 mole) and sodium bisulfite (600 mg.) in 500 ml. of water is added 57.6 g. of potassium cyanate and the solution is heated to 60° C. in a nitrogen atmosphere for one hour. Another 57.6 g. portion of potassium cyanate is then added and the heating continued for two hours. An NMR study indicated that at this point about 90% of the amino acid is converted to L-4-(3,4-dihydroxybenzyl)-4-methyl-hydantoic acid. This material is methylated without isolation as follows: water is distilled from the reaction mixture until ammonia is no longer detectable. The residue is diluted to the original volume, 20 ml. 8 N potassium hydroxide solution is added and cooled to 15° C. The solution is well agitated while 8 N potassium hydroxide solution (566 ml.) and dimethylsulfate (376 ml., 3.6 moles) are added simultaneously at such a rate to keep the temperature below 20° C. The addition takes about one hour. Half an hour later the mixture is extracted with ether. The extract contains a small amount of L-5-(dimethoxybenzyl)-3,5-dimethylhydantoin.

The aqueous layer is acidified to pH 2 with hydrochloric acid and the precipitated product is removed by filtration. After washing with water and drying, 79 g. of the hydantoic acid is obtained, 59.4% yield. An analytical sample is prepared by recrystallization from ethanol-water, m.p. 205–207° C.

Anal. calcd. for $C_{13}H_{18}N_2O_5$: C, 55.31; H, 6.43; N, 9.92. Found: C, 55.56; H, 6.52; N, 9.99.

B. Preparation of L- α -(3,4-dimethoxybenzyl)- α -hydrazino propionic acid

To an ice-cold solution of the hydantoic acid of procedure A (2.2 g., 7.8 mmoles) in 15.6 ml. of 2.5 N potassium hydroxide is added a solution of sodium hypochlorite (13.7 ml., 0.71 N, 9.75 mmoles). Five minutes after the addition is completed, the solution is heated to 80° C. for one and a half hours. After this period, toluene (45 ml.) and hydrazine hydrate (0.8 ml.) is added and the mixture is vigorously agitated while adding 8 ml. of concentrated hydrochloric acid. The mixture is stirred at 80° C. for 30 minutes, then the phases are separated and the aqueous layer extracted with 25 ml. of toluene. The toluene layer contains 3,4-dimethoxyphenylacetone and its condensation products. The aqueous layer is evaporated to dryness and the resulting salt mixture is digested with ethanol. The alcoholic solution is neutralized (pH 6.4) with diethylamine and the precipitated product is filtered, washed with ethanol and dried to afford 1.25 g. of L- α -(3,4-dimethoxybenzyl)- α -hydrazino-propionic acid, 60% yield.

An analytical sample is recrystallized from water; m.p. 222–224° C.

$$[\alpha]_D^{25} \text{AlCl}_3 \text{ sol.} -24.6$$

Anal. calcd. for $C_{12}H_{18}O_4 \cdot H_2O$: C, 52.93; H, 7.40; N, 10.29. Found: C, 53.01; H, 7.46; N, 10.28.

C. Preparation of L- α -methyl- β -(3,4-dihydroxyphenyl)- α -hydrazino propionic acid

A mixture of the L- α -(3,4-dimethoxybenzyl)- α -hydrazino propionic acid (10 g.) and concentrated hydrochloric acid (150 ml.) is heated in a sealed tube at 120° C. for 2 hours. The reaction mixture is evaporated to dryness *in vacuo* and the product is leached out with ethanol. The hydrazino acid is precipitated by the addition of diethylamine to pH 6.4. The precipitate is filtered,

washed with ethanol and dried, affording 6.5 g. of L- α -methyl- β -(3,4-dihydroxyphenyl) - α - hydrazino propionic acid (73%). Recrystallization from water (containing a small amount of sodium bisulfite) yields analytically pure material; m.p. 208° dec.

$$[\alpha]_{546}^{\text{AClCl}_3} -25.4^\circ$$

Anal. calcd. for $C_{10}H_{14}N_2O_4 \cdot H_2O$: C, 49.17; H, 6.60; N, 11.47.

Found: C, 49.13; H, 6.74; N, 11.19.

EXAMPLE 2

A. Preparation of L-4-(3,4-dimethoxybenzyl)-4-ethylhydantoic acid

The procedure of Example 1A is repeated employing L- α -amino- α -(3,4-dihydroxybenzyl)butyric acid rather than the alanine compound. The L-4-(3,4-dimethoxybenzyl)-4-ethylhydantoic acid is obtained in a yield of 70%. The product is recrystallized from ethanol-water to give a pure product having a melting point of 218–220° C.

Anal. calcd. for $C_{14}H_{20}O_5N_2$: C, 56.74; H, 6.80; N, 9.45. Found: C, 56.71; H, 6.88; N, 9.53.

B. Preparation of L- α -(3,4-dimethoxybenzyl)- α -hydrazinobutyric acid

The procedure of Example 1B is repeated employing the L-4-(3,4-dimethoxybenzyl) - 4 - ethylhydantoic acid to obtain a 53% yield of L- α -(3,4-dimethoxybenzyl)- α -hydrazinobutyric acid. An analytical sample is recrystallized from ethanol-water to give a pure product having a melting point of 215–220° C.

Anal. calcd. for $C_{13}H_{20}O_4N_2$: C, 58.19; H, 7.51; N, 10.44. Found: C, 58.16; H, 7.60; N, 10.40.

C. Preparation of L- α -hydrazino- α -ethyl- β -(3,4-dihydroxyphenyl)propionic acid

The procedure of Example 1C is repeated employing L- α -(3,4-dimethoxybenzyl)- α -hydrazinobutyric acid to give a 90% yield of L- α -hydrazino- α -ethyl- β -(3,4-dihydroxyphenyl)propionic acid which, when recrystallized from water-isopropanol, has a melting point of 209–212° C.

$$[\alpha]_D^{25} -15.2 \quad C=1$$

Anal. calcd. for $C_{11}H_{16}O_4N_2$: C, 54.99; H, 6.71; N, 11.66. Found: C, 55.02; H, 6.70; N, 11.65.

EXAMPLE 3

Testing for decarboxylase inhibition in mammals

Female albino mice weighing between 18 to 22 g. each are used. The animals are administered 80 mg./kg. of L-dopa (L-3,4-dihydroxyphenylalanine) in combination with the indicated dose of L- α -hydrazino- α -methyl- β -(3,4-dihydroxyphenyl)propionic acid orally in a solution or suspension in water. The animals are decapitated 90 minutes later. The brains are removed and pooled in groups of seven. Three separate pools are used for each drug treatment and the values obtained are averaged.

The brains are homogenized with 0.4 N perchloric acid, 9 ml. per gram of tissue. Catecholamines and catecholaminoacids are adsorbed onto and then eluted from alumina. The dopa and dopamine are separated by chromatography utilizing a column containing the ion exchange resin "Amberlite CG-50" with a size of 200–400 mesh. The dopa and dopamine are then subjected to iodine oxidation for the fluorimetric determination of dopa and

dopamine (Porter, C. C., Totaro, J. A. and Bercin, A., J. Pharmac. Exp. Therap. 150 17 (1965).

Control groups of mice are included and the average value for each of the three trials is given in Table 1.

TABLE 1

	Dosage, mg./kg.	Dopa, Mi- crograms/ gm.	Dopamine, micrograms/ gm.
Control.....		0.05	1.30
Racemate.....	20	3.60	3.05
L-form.....	10	2.85	2.68

As shown by the table, 10 mg. of the L compound has about the same activity as 20 mg. of the DL compound (racemate) in the test animals. In other words, the L form is essentially twice as active as the racemate in this test.

EXAMPLE 4

Comparison of the racemate, the D and the L isomers of α -hydrazino- α -methyl- β -(3,4-dihydroxyphenyl) propionic acid for their ability to potentiate the reversal by L-dopa of reserpine-induced suppression of locomotion and ptosis

The mice are housed in transparent plastic mouse boxes and acclimatized to their surroundings overnight. Various doses of the racemate, the L or the D isomers of α -hydrazino- α -methyl- β -(3,4-dihydroxyphenyl) propionic acid in methocel (suspending agent—1% methyl-cellulose in water) are administered orally one hour after the intraperitoneal administration of reserpine (4 mg./kg.). L-dopa (150 mg./kg.) is administered by the intraperitoneal route 2 hours after reserpine, and the mice observed, on a blind basis, for suppression of locomotion and the presence of ptosis one hour later. Suppression of locomotion is determined by placing the mice, individually, onto the center of an 8 x 10 inch wire-mesh grid for 15 seconds. If a mouse does not walk to or off the edge of the grid (normally occurring in less than 15% of reserpinized mice), locomotion is considered suppressed. Non-reserpinized mice invariably walk to or off the grid within this time period. Ptosis is graded positive if there is 50% or more closure of the eyelids.

TABLE 2

Comparison of the effect of the D and L isomers of α -hydrazino- α -methyl- β -(3,4-dihydroxyphenyl) propionic acid upon L-dopa antagonism of reserpine-induced suppression of locomotion and ptosis

	Dose (mg./kg. p.o.)	Reserpine-induced suppression of—	
		Locomo- tion	Ptosis
		Number mice protected/ Number mice tested	Number mice protected/ Number mice tested
Pretreatment: ^a Methocel.....		1/10	1/10
D isomer.....	5.0	1/10	1/10
plus methocel.....	25.0	2/10	2/10
	125.0	2/10	3/10
L isomer.....	0.2	3/10	2/10
plus methocel.....	1.0	5/10	7/10
	^b 5.0	8/10	9/10
ED ₅₀ ^c , mg./kg.....		0.86	0.56

^a One hour prior to L-dopa 150 mg./kg., i.p.

^b This dose of L- α -hydrazino- α -methyl- β -(3,4-dihydroxyphenyl) propionic acid was inactive as a reserpine antagonist when administered prior to methocel.

^c Estimated dose of α -hydrazino- α -methyl- β -(3,4-dihydroxyphenyl) propionic acid, when given in combination with L-dopa (150 mg./kg., i.p.), necessary to antagonize these effects of reserpine in 50% of the mice.

TABLE 3

Comparison of the effect of the racemate and the L isomer of α -hydrazino- α -methyl- β -(3,4-dihydroxyphenyl) propionic acid upon L-dopa antagonism of reserpine-induced suppression of locomotion and ptosis

	Dose (mg./kg., p.o.)	Reserpine-induced suppression of—	
		Locomo- tion	Ptosis
		Number mice protected/ Number mice tested	Number mice protected/ Number mice tested
Pretreatment: ^a Methocel.....		7/70	8/70
L isomer.....	0.07	4/30	5/30
Plus methocel.....	0.22	10/70	16/70
	0.67	20/70	23/70
	2.0	45/70	44/70
	6.0	33/40	34/40
ED ₅₀ ^b , mg./kg.....		1.2 (0.5-2.9)	1.0 (0.5-1.8)
Racemate plus methocel.....	0.67	11/70	10/70
	2.0	29/70	30/70
	6.0	49/70	50/70
	18.0	63/70	62/70
ED ₅₀ ^b , mg./kg.....		2.9 (2.4-3.5)	2.8 (2.2-3.8)

^a One hour prior to L-dopa 150 mg./kg., i.p.

^b Estimated dose of α -hydrazino- α -methyl- β -(3,4-dihydroxyphenyl) propionic acid, when given in combination with L-dopa (150 mg./kg., i.p.), necessary to antagonize these effects of reserpine in 50% of the mice. Values in parenthesis refer to the 95% confidence intervals.

Tables 2 and 3 show the effect of L-dopa (150 mg./kg. i.p.) upon reserpine induced suppression of locomotion and ptosis in mice pretreated with various doses of the racemate, the D and the L isomers of α -hydrazino- α -methyl- β -(3,4-dihydroxyphenyl) propionic acid. This dose of L-dopa (150 mg./kg. i.p.) is ineffective as a reserpine antagonist in mice pretreated with methocel. The doses of the racemate, the D and the L isomers of α -hydrazino- α -methyl- β -(3,4-dihydroxyphenyl) propionic acid necessary to antagonize the locomotive suppressant and ptosis actions of reserpine in 50% of the mice (ED₅₀), when given in combination with L-dopa (150 mg./kg.), are estimated from regression lines fitted to the data.

The D isomer of α -hydrazino- α -methyl- β -(3,4-dihydroxyphenyl) propionic acid shows little, if any ability to potentiate either of these effects of L-dopa in reserpinized mice (ED₅₀ > 125.0 mg./kg.). Comparison of the ED₅₀ for the racemate and the L isomer of α -hydrazino- α -methyl- β -(3,4-dihydroxyphenyl) propionic acid indicates that the L isomer (ED₅₀ 1.2 mg./kg.) is approximately 2.4 times as active as the racemate (ED₅₀ 2.9 mg./kg.) in potentiating L-dopa reversal of reserpine-induced suppression of locomotion. With regard to antagonism of reserpine-induced ptosis, the L isomer (ED₅₀ 1.0 mg./kg.) is found to be approximately 2.8 times as active as the racemate (ED₅₀ 2.8 mg./kg.).

EXAMPLE 5

To a solution of (10 millimoles) of L- α -hydrazino- α -methyl- β -(3,4-dihydroxyphenyl) propionic acid of Example 1 in 50 ml. of ethanol is introduced hydrogen chloride gas until saturated. The reaction mixture is stirred at room temperature for 24 hours and then evaporated to dryness. The crystalline ethyl ester hydrochloride is recrystallized from a mixture of ethanol and ethyl acetate. The ethyl ester is also useful in inhibiting decarboxylase.

Many other equivalent modifications of the invention would be apparent to those skilled in the art from a reading of the foregoing without a departure from the inventive concept.

What is claimed is:

1. A compound selected from the group consisting of (A) L - α - hydrazino - T - substituted - β -(3,4-dihydroxyphenyl)propionic acid substantially free of the D isomer, (B) the lower alkyl esters thereof and (C) the pharmaceutically acceptable salts thereof, wherein the substituent is lower alkyl.

2. L - α - hydrazino - α - methyl- β -(3,4-dihydroxyphenyl) propionic acid substantially free of the D isomer.

3. The pharmaceutically acceptable salts of the compound of Claim 2.

4. The lower alkyl esters of the compound of Claim 2.

5. L- α -hydrazino - α - ethyl- β -(3,4-dihydroxyphenyl)-propionic acid substantially free of the D isomer.

6. The pharmaceutically acceptable salts of the compound of Claim 5.

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LORRAINE H. WEINBERGER, Primary Examiner

L. A. THAXTON, Assistant Examiner

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