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(54) α-HYDRAZINO ACIDS

(71) 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:—

Certain α - hydrazino - α - substituted - β-(3,4 - dihydroxyphenyl) - propionic acids and their esters and salts form part of the invention of our prior Patent No 940596. It is known that racemates of these compounds are very potent decarboxylase inhibitors in mammals: see Sletzinger et al "Journal of Medicinal Chemistry", Volume 6, page 101 (1963) and Porter et al "Biochemical Pharmacology", Volume 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 compound is essentially inactive and may even be 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 and in other tests it appears that the *D* form will actually detract from the action of the *L* form.

The new compounds of the present invention are *L* - α - hydrazino - β - (3,4-dihydroxyphenyl)propionic acid substantially free of the *D* isomer, *L* - α - hydrazino - α-(C_{1-5} alkyl) - β - (3,4 - dihydroxyphenyl)-propionic acids, particularly *L* - α - hydrazine-α - methyl - β - (3,4 - dihydroxyphenyl)-propionic acid and *L* - α - hydrazino - ethyl-β - (3,4 - dihydroxyphenyl)propionic acid, substantially free of the *D* isomer, and their C_{1-5} alkyl esters, and pharmaceutically acceptable salts of the said acids and esters. Compounds of the present invention have been found to be much more potent decarboxylase inhibitors than were heretofore available to the medical profession. Such compounds have also been found to inhibit histidine decarboxylase, and thus to show promise of use as anti-histaminics as well.

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 be utilized only in the brain. Hydrazino compounds of the present invention have been found not to pass the blood-brain barrier and hence they inhibit decarboxylase only in the peripheral parts of the body. Thus, when *L*-dopa is used in conjunction with the hydrazino 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 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 overactivity in the production of serotonin from 5-hydroxytryptophane. The result of such overabundance 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

diarrhoea. Powerful decarboxylase inhibitors such as hydrazino compounds of this invention, especially those having no other physiological activity, are particularly suitable for such use.

The present invention also provides a method of inhibiting mammalian decarboxylase which comprises administering to a non-human mammal from 0.05 to 100 mg/kg per day of a compound of the invention.

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 of the invention. Such a composition may be in the form of a pill, tablet, capsule, syrup, elixir or injectable preparation.

The "pharmaceutically acceptable salts" of the compounds which may be used include the alkali metal and ammonium salts of the carboxy function and the hydrochloride, hydrobromide and sulfate salts of the amino function. Preferably the free base compounds are used, rather than the salts. The terminology "substantially free of the *D* isomer" signifies that less than 10% of *D* isomer is present. The C_{1-5} alkyl α-substituent or ester group preferably contains 1, 2 or 3 carbon atoms.

The free acids of the present invention are made by processes analogous to those set forth in Examples 1 to 3 below by choosing the appropriate *L* - α - amino - α - (3,4-dihydroxybenzyl)alkanoic acid starting material. The esters and salts are made by methods known from the literature as being suitable for forming such derivatives of amino acid. Example 6 sets out one such method.

The following examples, in which the words "Methocel" and "Amberlite" are Trade Marks, are given to illustrate the invention. All parts are given in parts by weight unless otherwise expressed.

Example 1

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

To a solution of *L* - α - methyl - β - (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. 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 the mixture cooled to 15°C. The solution is well agitated while 8 *N* potassium hydroxide solution (566 ml.) and dimethyl sulfate (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 - (3,4-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 - hydrazinopropionic 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 are 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 off, washed with ethanol and dried to afford 1.25 g. of *L* - α - (3,4 - dimethoxybenzyl) - α - hydrazinopropionic acid, 60% yield.

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

$AlCl_3$ sol.

$[\alpha]_D$ −24.6.

Anal. calcd. for $C_{12}H_{18}O_4.H_2O$: 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 off, 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.

$[\alpha]_{546}^{ACl Cl_3}$ −25.4°

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 using *L* - α - amino - α - (3,4 - dihydroxybenzyl)buytric 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 using 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 using *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.

$[\alpha]_D^{H_2O}$ −15.2 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

The procedure of Example 1A, 1B and 1C is repeated using *L* - β - (3,4 - dihydroxyphenyl)alanine as the starting material. The resulting product is *L* - α - hydrazino - β-(3,4 - dihydroxyphenyl)propionic acid.

Example 4

Testing for decarboxylase inhibition in mammalians

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 U.S. Standard 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 I

	Dosage Mg./Kg.	Dopa Micrograms/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* compounds (racemate) in the test animals. In other words, the *L* form is essentially twice as active as the racemate in this test.

EXAMPLE 5

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 (4mg./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 × 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 supression of locomotion and ptosis

Pretreatment[a]	Dose (mg/kg) p.o.)	Reserpine-induced Suppresion of Locomotion No. mice protected / No. mice tested	Reserpine-induced Supression of Ptosis No. mice protected / No. mice tested
Methocel	—	1/10	1/10
D isomer	5.0	1/10	1/10
+ Methocel	25.0	2/10	2/10
	125.0	2/10	3/10
L isomer	0.2	3/10	2/10
+ Methocel	1.0	5/10	7/10
	5.0[b]	8/10	9/10
	ED_{50}[c]	0.86 mg/kg	0.56 mg/kg

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-hydrozine-α-methyl-β-(3,5-dihydroxyphenyl) propionic acid upon *L*-dopa antagonism of reserpine-induced supression of locomotion and ptosis

Pretreatment[a]	Dose (mg/kg) p.o.)	Reserpine-Induced Supression of Locomotion No. mice protected / No. mice tested	Reserpine-Induced Suppression of Ptosis No. mice protected / No. mice tested
Methocel	—	7/70	8/70
L isomer	0.07	4/30	5/30
+ 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]	1.2 mg/kg (0.5—2.9)	1.0 mg/kg (0.5—1.8)
Racemate	0.67	11/70	10/70
+ Methocel	2.0	29/70	30/70
	6.0	49/70	50/70
	18.0	63/70	62/70
	ED_{50}[b]	2.9 mg/kg (2.4—3.5)	2.8 mg/kg (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 (ED_{50}), 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 ($ED_{50} > 125.0$ mg/kg). Comparison of the ED_{50} for the racemate and the *L* isomer of α-hydrazino - α - methyl - β - (3,4 - dihydroxyphenyl) propionic acid indicates that the *L* isomer (ED_{50} 1.2 mg/kg) is approximately 2.4 times as active as the racemate (ED_{50} 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 (ED_{50} 1.0 mg/kg) is found to be approximately 2.8 times as active as the racemate (ED_{50} 2.8 mg/kg).

EXAMPLE 6

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 the solution is saturated with hydrogen chloride. 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.

WHAT WE CLAIM IS:—

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

2. *L*- α - hydrazino - α - C_{1-5} alkyl) - β-(3,4 - dihydroxyphenyl)propionic acids substantially free of the *D*-isomer.

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

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

5. The C_{1-5} alkyl esters of a compound claimed in any one of claims 1—4.

6. A pharmaceutically acceptable salt of a compound claimed in any one of claims 1—5.

7. The preparation of a compound as claimed in any one of claims 1—6 by a process substantially as hereinbefore described in any appropriate Example.

8. A compound as claimed in any one of claims 1—6 when made by a preparation as claimed in claim 7 or its obvious chemical equivalent.

9. A method of inhibiting mammalian decarboxylase that comprises administering to a non-human mammal from 0.05 to 100 mg/kg per day of a compound as claimed in any one of claims 1—6 and 8.

10. A pharmaceutical composition comprising an inert pharmaceutical carrier and from about 5 mg to about 15 g of a compound as claimed in any one of claims 1—6 and 8.

11. A composition as claimed in claim 10 in the form of a pill, tablet, capsule, syrup, elixir or injectable preparation.

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