

PATENT SPECIFICATION

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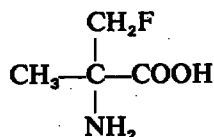
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(54) FLUORINATED AMINO ACIDS

(71) We, MERCK & CO. INC., a Corporation duly organized 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:—

The present invention is concerned with substituted α - fluoromethyl - α - amino alkanolic acids.

An unsubstituted α - fluoromethyl - α - amino alkanolic acid, namely 2-fluoromethylalanine, which has the formula:



(A)

is known [Kellonitsch *et al.*, J. Org. Chem. 40, 3808—9 (1975)]. No specific biological activity for this compound is suggested. This compound (A) is prepared by fluorodehydroxylation of the corresponding 2-hydroxymethylalanine.

α -Methyl amino acids, such as L - α - methyl - 3,4 - dihydroxyphenylalanine (α -methyldopa, an antihypertensive agent), are known to have decarboxylase-inhibiting activity (Goodman, *et al.*, *The Pharmacological Basis of Therapeutics*, MacMillan Company, New York, New York 1970, p. 577; Canadian Patent 737,907).

The present invention is based on the discovery of certain novel substituted α - fluoromethyl - α - amino alkanolic acids, at least some of which have decarboxylase-inhibiting activity significantly greater than that of α -methyl amino acids.

The present invention provides compounds having the formula:

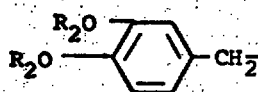


where R is a substituted C₁—C₄ alkyl group and R₁ is H or C₁—C₁₈ alkyl.

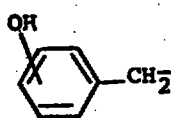
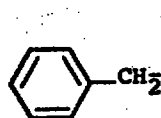
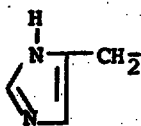
Compounds that are pharmaceutically acceptable acid-addition salts of the formula I compounds are also included. In general, the salts are those of the formula I base with a suitable organic or inorganic acid. Preferred inorganic acid salts are the hydrohalides, e.g., hydrochlorides, hydroiodides and hydrobromides; the sulfates, and the phosphates. The hydrohalides, and especially the hydrochlorides, are especially preferred.

The formula I compounds have a chiral center and may occur in optically active forms, i.e., as optical isomers. These isomers are designated conventionally by the symbols L and D, + and -, l and d, S and R or combinations thereof. Where the compound name or formula has no isomer designation, the name or formula includes the individual isomer mixtures and the racemates.

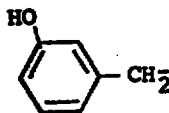
The compounds having the S-isomer configuration are, in general, preferred. R is a substituted alkyl group exemplified by



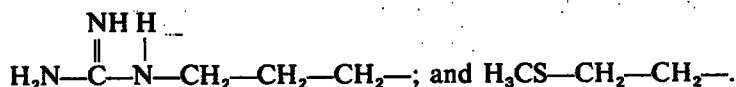
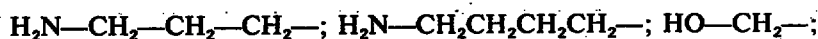
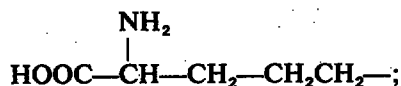
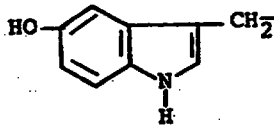
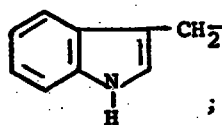
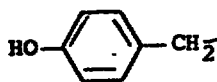
where R_2 is H or C_2-C_8 alkanoyl, e.g., CH_3-CO , $CH_3(CH_2)_4-CO$ or $(CH_3)_3C-CO$,



preferably

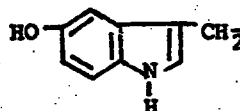
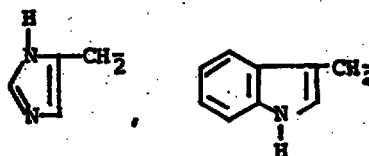
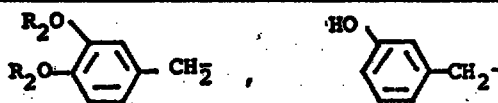


or



R_1 is H, which is preferred, or C_1-C_8 alkyl. Examples of suitable alkyl groups are methyl, octadecyl, 2-ethylhexyl, t-butyl, hexyl, isopropyl, ethyl and undecyl; C_1-C_6 alkyl is preferred and ethyl is especially preferred.

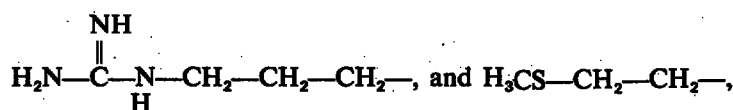
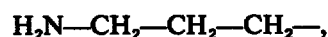
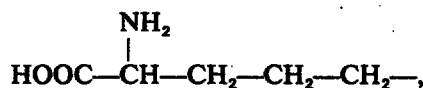
Preferred compounds of formula I are those where R is



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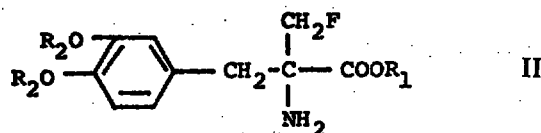


especially where R_1 is hydrogen.

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Compounds that are particularly preferred have the formula

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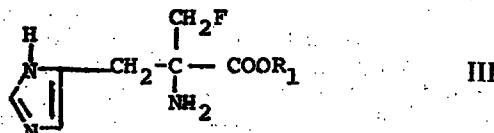


especially where R_2 is hydrogen and R_1 is hydrogen or ethyl. Especially preferred formula II compounds are those where R_1 and R_2 are hydrogen, with the S-isomer configuration being particularly preferred.

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Another particularly preferred compound has the formula

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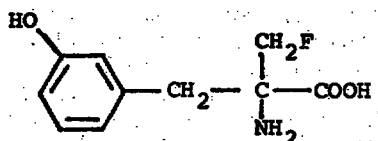


especially where R_1 is hydrogen.

The S-isomer of formula III is particularly preferred.

Especially preferred compounds are those of the formulae

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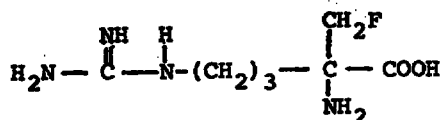
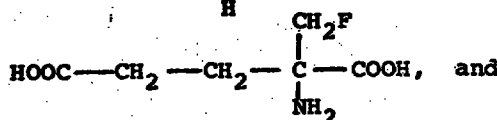
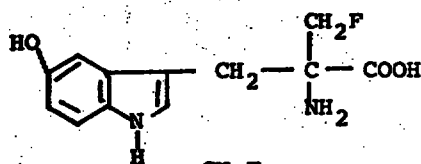
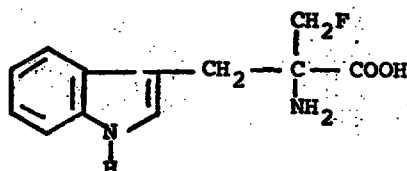
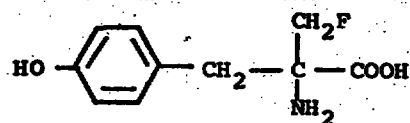
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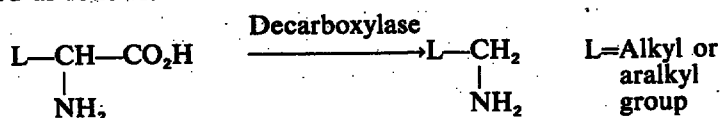
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The compounds of the present invention have physiological or chemotherapeutic uses. In most cases, the biological activities of these compounds are in large measure a consequence of their potent decarboxylase inhibiting activities. Decarboxylases are enzymes which act on α -amino acid substrates, effecting decarboxylation to produce the corresponding amine. This action is illustrated as follows:

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α -amino acid substrate

amine

L=Alkyl or aralkyl group

By inhibiting this decarboxylation, the biosynthetic pathway to a number of biologically significant amines can be modulated or inhibited with physiologically useful consequences. For example, α -fluoromethyl dopa inhibits dopa decarboxylase and can be used in combination with dopa to potentiate the latter's usefulness in the treatment of Parkinson's disease. α -Fluoromethyl-histidine inhibits biosynthesis of histamine via decarboxylation of histidine (ED_{50} in mice ~ 0.4 mg/kg). Consequently, it and combinations with histamine antagonists have utilities in the prevention of gastric lesions and in treating allergic conditions. α -Fluoromethyl ornithine by virtue of its ornithine decarboxylase inhibition interrupts polyamine biosynthesis and is of utility in the treatment of some neoplasms. α -Fluoromethyl-arginine is an effective antibacterial. α -Fluoromethyl-glutamic acid is a CNS stimulant.

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The present compounds are also substantially specific in their decarboxylase inhibition activity, that is an α -fluoromethyl- α -amino acid generally inhibits the decarboxylation of the corresponding non α -fluoromethyl acid. For example, α -fluoromethyl dopa inhibits the decarboxylation of dopa; α -fluoromethyl histidine will inhibit the decarboxylation of histidine, etc.

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Because of this specificity and potency as decarboxylase inhibitors, the present compounds are also useful as diagnostic tools to determine the presence and importance of the corresponding decarboxylase in relation to diseases or to the

functioning of biological systems. For example, the importance of γ -amino-butyric acid in the central nervous system (CNS) may be studied by inhibiting its biosynthesis using an α -fluoro-methyl-glutamic acid. This diagnostic utility is aided by the potent and in many instances irreversible decarboxylase inhibiting activity of the present α -fluoromethyl amino acids.

Representative compounds have been determined to have decarboxylase-inhibiting activity using conventional *in-vitro* assays.

α - Fluoromethyl - 3,4 - dihydroxyphenylalanine, α -fluoromethyl tyrosine, and α -fluoromethyl-meta-tyrosine have also been found to have antihypertensive activity. This activity is determined by observing the antihypertensive effect (blood pressure reduction) on (peroral or parenteral) administration of the compounds to a spontaneously hypertensive (SH) rat. This observed effect indicates that the compounds are effective as antihypertensive agents, when conventionally administered in suitable amounts in an appropriate pharmaceutical dosage form to a hypertensive human. The pharmaceutical dosage form is conventionally prepared and generally includes pharmaceutically acceptable diluents.

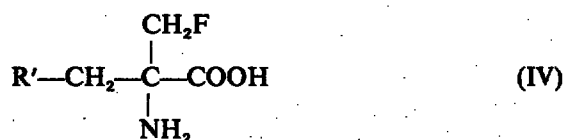
The compounds of the present invention may be prepared using any convenient method.

One such useful process involves the reaction of an α - hydroxymethyl - α - amino acid with SF_4 in liquid HF, as illustrated by the following equation:

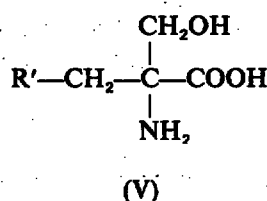


The reaction is generally carried out at temperatures in the range -80°C to 20°C . This general reaction is also referred to as fluorodehydroxylation and is described in the Journal of Organic Chemistry 40, 3809-10 (1975). BF_3 may be used to promote the reaction.

It has now been discovered that the fluorodehydroxylation of certain aryl-substituted α - hydroxymethyl - α - amino acids is substantially improved by utilizing BF_3 or AlCl_3 as a co-reactant with SF_4 . Specifically, this is an improved process for preparing a compound having the formula

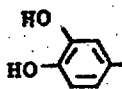


where R' is an aryl group, that comprises reacting a compound having the formula

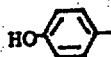


with a) SF_4 and b) BF_3 or AlCl_3 , in liquid HF at temperatures in the range -80°C to 20°C .

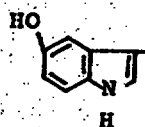
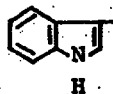
R' is an aryl group exemplified by



preferably



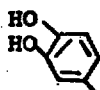
or



and



Preferred R' groups are



and



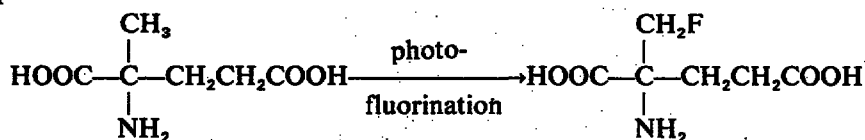
The compounds of formulae IV and V preferably have the S-isomer configuration.

This present process is preferably carried out at atmospheric pressure although pressures above atmospheric may be used. The reaction temperature ranges from -80°C to 20°C , -80°C to 0°C being preferred.

The present process may conveniently be carried out by introducing the SF_4 and BF_3 or AlCl_3 into the Formula V/HF reaction system initially. The process may also be carried out by first adding the SF_4 to the reaction system, allowing the reaction to proceed for a period of time and then adding the BF_3 or AlCl_3 and allowing the reaction to go to completion.

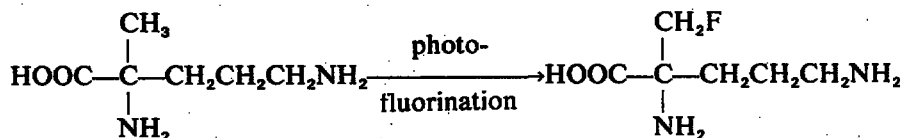
The use of BF_3 or AlCl_3 in the SF_4 /HF reaction system substantially improves the yield of Formula IV product.

Another method for preparation of the substituted α -fluoromethyl- α -amino alkanolic acids involves the application of photofluorination. For a description of this method, see *Journal of the American Chemical Society*, 92, 7494 (1970) and *ibid.*, 98, 5591 (1976). For example, α -fluoromethyl-glutamic acid is prepared:



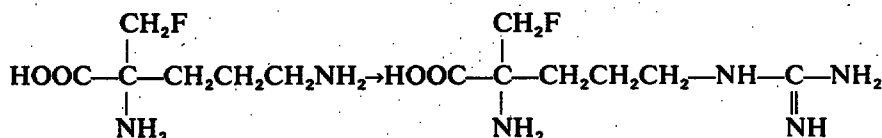
Both optical isomers of α -methylglutamic acid are known; thus this method is useful for preparation of both optical isomers of α -fluoromethylglutamic acid.

Similarly, α -fluoromethyl-ornithine is prepared by photofluorination of α -methyl-ornithine:



Since both optical isomers of α -methylornithine are available, this method of synthesis can deliver both of the two optical isomers of α -fluoromethyl-ornithine.

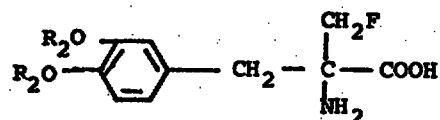
α -Fluoromethyl-ornithine is a suitable starting material for synthesis of α -fluoromethyl-arginine by reaction with S-methylisothiourea:



An acid-addition salt of the present invention may be prepared by conventional treatment of the free α -amino acid with a useful acid generally in a suitable solvent.

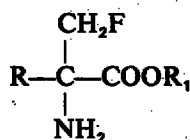
A single enantiomer of the present compounds may also be obtained by (1) resolving the fluorinated amino acid racemate using conventional resolution techniques or (2) resolving the precursor α -hydroxymethyl- α -amino acid using conventional resolution techniques and then fluorodehydroxylating the precursor enantiomer. A conventional resolution technique involves forming a salt of the α -amino acid with an optically active base and subsequently recovering the specific enantiomer from the salt.

Compounds of the formula



where R_2 is C_2 — C_8 alkanoyl are prepared by acylating the corresponding compound where R_2 is hydrogen. Conventional acylating agents and conditions are used.

Compounds of the formula

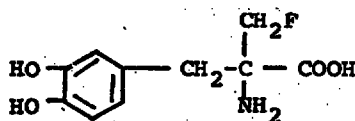


where R_1 is C_1 — C_{18} alkyl are prepared by esterifying the corresponding compound where R_1 is hydrogen. Again, conventional esterification reagents and conditions are employed.

The following examples illustrate preparation of representative compounds of the present invention. All temperatures are in $^{\circ}\text{C}$. The fluorodehydroxylation reactions described in the examples were performed in reactors made of KEL-F. Melting points are determined in open capillary and are uncorrected. The words 'KEL-F', 'Dowex', 'Celite' and 'Darco' are Trade Marks.

EXAMPLE 1

Preparation of R,S- α -(Fluoromethyl)-3-Hydroxy-Tyrosine



1.5 g of R,S- α -(hydroxymethyl)-3-hydroxytyrosine hydrochloride (α -hydroxymethyl-DOPA HCl) was dissolved in 50 ml of anhydrous hydrogen fluoride, while being cooled in a dry-ice-acetone bath. The HF solvent was then evaporated after removal of the cooling bath with a stream of nitrogen gas. This operation transformed the HCl salt into the HF salt of the starting material. (Alternatively 1.3 g of the free amino acid may be used as starting material, thus eliminating the need for the above operation). The HF salt thus obtained was redissolved by passing into the reactor a stream of HF gas after cooling it in a dry-ice-acetone bath, until 30 ml of liquid HF had been collected in the reactor. Sulfur tetrafluoride gas (1.2 ml, measured in liquid state at -78°C) was then passed in and the dry-ice-acetone cooling bath was then removed and replaced by a cooling bath kept at -12°C . After 15 hours of aging, the solvent was evaporated with a stream of N_2 , the residue was dissolved in 50 ml of 2.5 M aqueous HCl, evaporated to dryness *in vacuo* and subjected to amino acid analysis on Spinco-Beckman amino acid analyser. This analysis indicated the formation of α - fluoromethyl - 3 - hydroxy - tyrosine. The product R,S - α - fluoromethyl - 3 - hydroxy - tyrosine is isolated by ion-exchange chromatography in the same manner as it is described in Example 2 for S - α - fluoromethyl - 3 - hydroxy - tyrosine.

EXAMPLE 2

Preparation of S- α -fluoromethyl-3-Hydroxy-TyrosineA.) Preparation of R- α -hydroxymethyl-3-hydroxy-tyrosine

50 g of 3 - (3',4' - diacetoxyphenyl) - 2 - acetamido - 2 - acetoxymethyl - propionic acid is added to 204 ml of 4 M aqueous KOH with stirring. After 1 hour of stirring (under nitrogen), the solution contains the potassium salt of 3 - (3',4' - dihydroxyphenyl) - 2 - acetamido - 2 - hydroxymethyl - propionic acid, formed in essentially quantitative yield. Without isolation, by methylation with dimethyl sulfate, this compound is transformed into 3 - (3',4' - dimethoxyphenyl) - 2 - acetamido - 2 - hydroxymethyl - propionic acid. This operation is performed at room temperature under N₂ gas by dropwise addition with vigorous stirring of dimethyl sulfate (about 64 ml) and 4 M aqueous KOH solution (about 148 ml) over a period of about 1 hour.

The reaction mixture was stirred for another hour, then left standing overnight. Acidification (at 5—10°C with 55 ml of conc. aqueous HCl), extraction with ethyl acetate (12×300 ml), drying over Na₂SO₄ and evaporation *in vacuo* gave R,S - 3 - (3',4' - dimethoxyphenyl) - 2 - acetamido - 2 - hydroxymethyl - propionic acid. It was purified by recrystallization from 1325 ml of acetonitrile, m.p. 154—6°C (dec).

29.1 g of strychnine was suspended in 1.12 l of ethanol, heated to reflux, then 26.1 g of R,S - 3 - (3',4' - dimethoxyphenyl) - 2 - acetamido - 2 - hydroxymethyl - propionic acid was added. The solution thus obtained was allowed to cool down and left standing overnight at room temperature. Crystals of the strychnine salt of antimer, "A" separate; m.p. 193—194°C ("HM").

The mother liquor of the above named precipitation was evaporated *in vacuo* to dryness and recrystallized from 270 ml of ethanol; the hot solution is allowed to cool to room temperature and left standing at room temperature for about 3 hours, then kept in the refrigerator for about 4 hours. The crystals formed were collected on a filter and after drying, recrystallized from acetonitrile to give the strychnine salt of antimer "B" of 3 - (3',4' - dimethoxy - phenyl) - 2 - acetamido - 2 - hydroxymethyl - propionic acid, m.p. 130—132°C (dec.), yield 17.5 g.

17 g of this strychnine salt was decomposed by dissolving it first in 160 ml of water; 31 ml of 1 M aq. NaOH solution was added. The strychnine separated was removed by filtration and the solution evaporated to a small volume *in vacuo* and applied onto a small ion exchange resin column (150 ml of AG—X2 cation exchange Dowex 50 resin, 200/400 mesh). Elution with water, followed by evaporation *in vacuo* of the fractions which showed absorption, as indicated by an LKB UV absorption monitor (Uvicord II-8300). This compound, antimer "B" of 3 - (3',4' - dimethoxyphenyl) - 2 - acetamido - 2 - hydroxymethyl - propionic acid showed $[\alpha]_D^{25}$: 78.3+0.5° (C, 1.425 in 0.1 M aq. NaOH).

Transformation of the above compound into the corresponding stereo-isomer of α - hydroxymethyl - 3 - hydroxytyrosine: 4.43 g of antimer "B" of 3 - (3',4' - dimethoxyphenyl) - 2 - acetamido - 2 - hydroxymethylpropionic acid is dissolved in 100 ml conc. HCl and sealed and heated for 90 minutes in a Fisher-Porter tube immersed into an oil bath of 130°C. The solvent is evaporated *in vacuo* and the above HCl treatment repeated. The residue thus obtained represents R - α - hydroxymethyl - 3 - hydroxy - tyrosine hydrochloride.

B.) Fluorodehydroxylation

8 g of R - α - hydroxymethyl - 3 - hydroxy - tyrosine. HCl is charged to a 1 l reactor. The reactor is immersed in a dry-ice acetone bath and 80 ml of liquid HF is condensed on top of the substrate. To remove the HCl present, the cooling bath is removed and the HF solvent removed by passing in a stream of N₂ gas. The reactor is immersed in the cooling bath again and a stream of HF gas is passed in until a liquid volume of about 250 ml collects. 6.2 ml of SF₄ (17.6 mmol/ml: about 109 mmol) is then bubbled in, the solution aged for about 1 hour, the cooling bath exchanged for an ethylene-glycol bath kept at -16°C and the solution aged for about 22 hours. Boron trifluoride gas is passed in until saturation and the solution aged again at -16°C for 46 hours. The cooling bath is removed and the solvent evaporated by passing through it a vigorous stream of N₂ gas. The residue is quenched in about 100 ml of ice-cold aqueous HCl (2.5 M), evaporated *in vacuo*, the residue dissolved in water and added onto a column of 2.2 l of Dowex AG-50-X-8 cation-exchange resin (200/400 mesh). Elution with 0.25 M aq. HCl, containing 5% methanol; in ~8.5 hours, 7.2 l of this solvent is pumped through the column. This is followed by 7.2 l of 0.4 M aq. HCl with 7.5% methanol in 8.5 hours, then

concluding with 0.6 M aq. HCl with 10% methanol. 22 ml fractions are collected, 10 tubes per rack. Tubes in racks No. 45—66 contained the desired compound. Evaporation in vacuo gave the HCl salt of the S-isomer of α - fluoromethyl - 3 - hydroxy - tyrosine.

For liberation of the free amino acid, 4.826 g of this compound was dissolved in 90 ml of isopropanol and filtered through Celite. 6.2 ml of propylene oxide was added to the filtrate and the suspension kept at room temperature for 3.5 hours, then at about 5°C for another 2.5 hours. The S - α - fluoromethyl - 3 - hydroxy - tyrosine thus formed was collected by filtration, washed with isopropanol and dried overnight in vacuo at 76°C. $[\alpha]_D^{25}$: +9.3° ± 0.5, c, 1.82 in 1:1 mixture of trifluoroacetic acid and water.

EXAMPLE 3

Preparation of R- α -Fluoromethyl-3-Hydroxy-Tyrosine

For preparation of the above named compound, the strychnine salt of antimer A of 3 - (3',4' - dimethoxyphenyl) - 2 - acetamido - 2 - hydroxymethyl - propionic acid (Example 2 "HM") was carried through steps analogous to those in Example 2. The final product of the sequential steps was R - α - fluoromethyl - 3 - hydroxy - tyrosine, with $[\alpha]_D^{25}$: -9° (c, 2.5 in a 1:1 mixture of H₂O-trifluoroacetic acid).

EXAMPLE 4

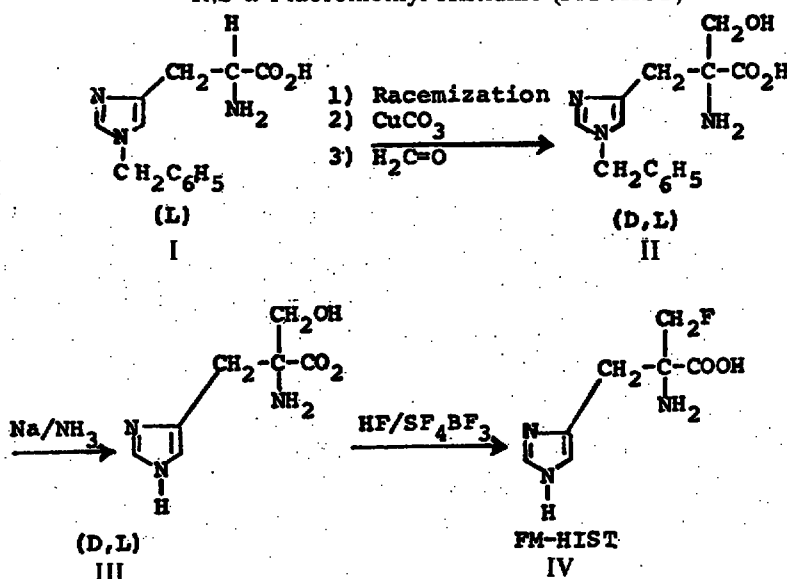
R,S- α -Fluoromethyl-Tyrosine

1.05 g (0.005 mol) of R,S - α - hydroxymethyl - tyrosine is charged into a reactor. The reactor is immersed in a dry-ice-acetone bath and about 50 ml of liquid HF is collected by passing in a stream of HF gas. Under continuing cooling, SF₄ gas (4 ml, measured in liquid state at -78°C) is passed in, then BF₃ gas until saturation at -78°C. (Stirring with magnetic stirrer). The deep-red solution thus obtained is aged overnight at -78°C; the cooling bath is removed then, and the solvent evaporated by blowing a dry stream of nitrogen gas through it.

The residue is dissolved in 20 ml of 2.5 M aq. HCl and evaporated to dryness in vacuo. The residue is dissolved in water and applied to a strong acid cation-exchange resin column, prepared with 100 ml of Dowex AG50-X-8 resin (200/400 mesh). The column is first washed with water (1.8 l), followed by 0.5 M aq. HCl. 20 ml fractions of the effluent are collected and the course of the elution is followed by an UV monitor of LKB, Model Uvicord II. The fractions corresponding to the main peak in the UV curve are combined and evaporated to dryness in vacuo, to yield the hydrochloride salt of R,S-fluoromethyl-tyrosine. 400 ml of this salt is dissolved in 6 ml of water; after a few minutes, crystallization of R,S - α - fluoromethyl - tyrosine begins. After standing overnight at 5°C, the product is filtered, washed with water, ethanol and diethyl ether and dried in vacuo at 76°C, to give R,S - α - fluoromethyl - tyrosine.

EXAMPLE 5

R,S- α -Fluoromethyl-Histidine (FM HIST)



A) Racemic N(im)Benzyl-Histidine

30 g of N(im)Benzyl-L-histidine is dissolved in 600 ml H₂O and the solution heated in a high-pressure autoclave at 200°C for 8 hours with shaking. The autoclave is cooled to room temperature, the clear supernatant solution evaporated in vacuo to dryness to give the racemic N(im)benzyl-histidine as a colorless crystal.

B) R,S- α -Hydroxymethyl-Histidine Dihydrochloride

20 g of racemic N(im)benzyl-histidine is dissolved in 1 l of hot water, then 40 g of basic cupric carbonate is added in portions and the mixture refluxed with stirring for 1 hour. The mixture is filtered while hot and the filtrate is evaporated in vacuo to give the Cu chelate of racemic N(im)benzyl-histidine as a blue solid.

A mixture of 31 ml of formalin (38% H₂CO), 3.1 ml of pyridine and 2.13 g of Na₂CO₃ is heated with stirring to 70°C, then 20 g of the above named Cu chelate is added and the system heated and stirred at 75°C for 90 minutes. Evaporation *in vacuo* gives a blue solid residue. This is dissolved in a mixture of 50 ml of H₂O with 50 ml of conc. NH₄OH and charged onto a cation-exchange resin column (Dowex 50-X-8, 300 ml resin in the NH₄-form) and eluted with 2 M aq. NH₄OH solution. The effluent is monitored with a LKB Uvicord II UV absorption monitor and 1.1 l of the effluent is found to have UV absorption. This portion is evaporated *in vacuo* to a solid. The residue is dissolved in a mixture of 60 ml of H₂O with 5 ml of conc. aq. NH₄OH and charged onto an anion-exchange resin column (300 ml of Dowex 1-X-2 resin in the OH⁻ form). The column is washed with water (2 l), and eluted with 2 M aq. HCl and monitored with a Uvicord II for UV absorption. The effluent fractions with ultraviolet absorption are combined and evaporated to dryness, to give substantially pure HCl salt of N(im)benzyl - α - hydroxymethyl - histidine (II) (new compound). This compound is transformed into α - hydroxymethyl - histidine (III) in the following way: 12.5 g of II is dissolved in 200 ml of liquid NH₃ (3-neck flask, equipped with "cold-finger" condenser filled with dry-ice-acetone), then sodium is added (5.5 g, cut in small pieces) until the blue color persists for 10 minutes. HN₄Cl is added then to consume the excess of Na (indicated by decolorization) and the NH₃ solvent is allowed to evaporate under a stream of N₂. The product III thus obtained is purified by chromatography on a cation-exchange resin column (2.2 l of Dowex-50-X-8, 200/400 mesh). Crude III is dissolved in 100 ml of H₂O and applied onto the resin column. The column is washed first with water (4 l) and developed with aq. HCl (1.5 M, then 2 M). 20-ml fractions are collected, flow rate 600 ml/hr.

Fraction No.

1—400
401—670
671 & later

1.5 M HCl
2 M HCl

Pauly Reaction

—
—
+

Fractions 671—760 are combined and evaporated in vacuo to dryness, to give R,S - α - hydroxymethyl - histidine · 2 HCl (new compound).

C) R,S- α -Fluoromethyl-Histidine (IV)

2.73 g of R,S - α - hydroxymethyl - histidine · 2 HCl is dissolved in 70 ml of liquid HF and then evaporated to dryness by passing in a stream of N₂. The residue thus obtained represents the hydrofluoride salt of α -hydroxymethyl-histidine. It is redissolved in 200 ml of liquid HF (dry-ice-acetone cooling bath), then 9 ml SF₄ is passed in (measured as liquid at -78°C). The solution is stored overnight, while being kept in a cooling bath of -12°C. The solution is saturated then with BF₃ gas, left standing for 5 hours, saturated again at -12°C and left aging at the same temperature for 66 hours. The cooling-bath is then removed and the solvent evaporated by passing in a stream of N₂. The residue represents mainly the HBF₄ salt of α -fluoromethyl-histidine. This is dissolved in 100 ml of 2.5 M aq. HCl, evaporated to dryness and transformed into the HCl salt as follows: It is redissolved in H₂O and applied onto a cation-exchange resin column (100 ml of Dowex AG50-X-2, 200/400 mesh) and eluted with H₂O until effluent is neutral and free of F⁻. The product is released from the column by 3 M aq. HCl, evaporated to dryness *in vacuo*, to result in a residue consisting mainly of the dihydrochloride of IV. For final purification, this is rechromatographed on another AG-50-X-2 column (900 ml resin).

Elution with: 0.5 M aq. HCl — 1 l.
1.0 M aq. HCl — 1.5 l.
1.5 M aq. HCl — 3.3 l (collection
begins here,
20-ml fractions)
2.0 M aq. HCl — 8.00 l.

The desired product IV is located by Pauly test. Fractions 390—470 are combined and evaporated to dryness *in vacuo* to give pure dihydrochloride of IV. Recrystallization from water-isopropanol (1:9 v/v) gives the crystalline monohydrochloride salt of α -fluoromethyl-histidine, m.p. 226—7° (dec).

EXAMPLE 6

Synthesis of *R,S*- α -Fluoromethyl-Ornithine

A) *R,S*- α -Hydroxymethyl- δ -N-Benzoyl-Ornithine

The copper chelate of *R,S* - δ - N - benzoyl - ornithine (7.995 g) is added in small portions onto a mixture made of formalin (38% H₂CO; 12.45 ml), pyridine (1.25 ml), and sodium carbonate (0.81 g) at 70°C, under mechanical stirring. After a further 90 minutes' stirring at 75°C, it is evaporated to dryness *in vacuo*, the dark blue residue dissolved in a mixture of 30 ml of H₂O and 30 ml of conc. aq. NH₃ solution and charged to a cation-exchange resin column (130 ml of Dowex 50-X-8 in the NH₄⁺ form) to remove Cu²⁺. The column is eluted with 250 ml of 2 M aq. NH₃ and the effluent evaporated to dryness *in vacuo*. The residue is redissolved in H₂O and applied onto an anion exchange resin column (Dowex 1-X-2, OH⁻ form, 130 ml resin). The column is washed with H₂O (250 ml) and eluted with 3 M aq. HCl. The HCl effluent is concentrated *in vacuo* to give *R,S* - α - hydroxymethyl - δ - N - benzoyl - ornithine.

B) *R,S*- α -Hydroxymethyl-Ornithine Dihydrochloride

3.5 g of the product obtained in (A) is dissolved in 40 ml of 6 M aq. HCl and refluxed for 21 hours. The solution is extracted with toluene (2×40 ml) and the aqueous phase evaporated *in vacuo* to dryness, to give *R,S* - α - hydroxymethyl - ornithine dihydrochloride (new compound).

C) *R,S*- α - Fluoromethyl-Ornithine

1.1 g of the product obtained under (B) is placed in a reactor, the reactor immersed into a dry-ice-acetone bath and HF gas passed in until HF solution of 25 ml volume is formed in the reactor. The cooling bath is removed and the solvent evaporated by passing in a stream of N₂. The residue thus obtained represents the HF salt of *R,S* - α -hydroxymethyl - ornithine. This residue is redissolved in HF, by cooling the reactor in the dry-ice-acetone bath and passing in HF gas until 50 ml volume is reached. SF₄ gas is passed in (4 ml as measured in liquid state at -78°C), the dry-ice-acetone cooling bath removed and replaced by a bath kept at -15°C. After aging for 16 hours at -15°C, BF₃ gas is passed in for saturation. After 5 hours further aging, the cooling bath is removed and the solvent evaporated by passing in a stream of N₂. The residue is dissolved in 6 M aq. HCl, evaporated to dryness *in vacuo* and redissolved in H₂O (10 ml). This solution is applied onto a Dowex 50-X-8 cation-exchange resin column (400 ml resin, 200/400 mesh, H⁺ form). The column is first washed with H₂O (800 ml); elution with 2 M aq. HCl, 15 ml fractions are collected. Flow rate 600 ml/h. Every 5th fraction is spotted on TLC plate and developed with ninhydrin spray. Fractions No. 171—220 are combined and evaporated to dryness *in vacuo*, to deliver a mixture of amino acids, the main component being *R,S* - α - fluoromethyl - ornithine · 2 HCl. For further purification, this product is rechromatographed on another column, made of Dowex 50-X-8 cation exchange resin (200/400 mesh). For development, the column is first washed with water, then eluted with 1.5 aq. HCl, flow rate 0.6 l/h. 20-ml fractions are collected. The residue obtained on evaporation of fractions No. 521—540 represents pure *R,S* - α - fluoromethyl - ornithine dihydrochloride.

EXAMPLE 7

Synthesis of *S*- α -Fluoromethyl-Tyrosine

A.) Preparation of the Copper Chelate of Tyrosine Methyl Ether

25 g of *R,S*-tyrosine methyl ether (128 mmol) was dissolved in 646 ml of 0.2 N NaOH at 80°C and this solution was added to 16.1 g of copper sulfate pentahydrate dissolved in 1600 ml of water at 80°C. An immediate precipitate was formed and the solution was allowed to cool overnight, after which it was filtered affording 28.9 g of the copper chelate of *R,S*-tyrosine methyl ether.

B) R,S- α -Hydroxymethyl Tyrosine Methyl Ether

29 g of the copper (Cu^{++}) chelate of tyrosine methyl ether (0.064 mole) was added at 70°C under stirring to a solution of 3.9 g of sodium carbonate, 52 ml of 37% aqueous formaldehyde and 5.2 ml of pyridine (nitrogen blanket). After completion of addition, there was added another 18 ml of formaldehyde solution and 1.6 ml of pyridine. After heating at 70°C for 3.5 hr and allowing the solution to cool to room temperature in an additional 1.5 hr, the solution remained at room temperature overnight. In the morning, there appeared copious blue crystals which were filtered off and the filtrate concentrated to dryness *in vacuo*. After the residue had been dissolved in water and reconcentrated to dryness, it was dissolved in 90 ml of 4 N HCl. After filtration the solution was used to dissolve the above blue crystals. This required an additional 300 ml of 4 N HCl. The solution was then treated with hydrogen sulfide, filtered through a diatomaceous earth filter aid and concentrated to about 40 g of crude product. This was applied to a strong acid cation-exchange resin (0.5% of Dowex 50-X-8), eluted with 4 l of water and then 2 N aqueous ammonia. The effluent was monitored with Uvicord II (recording ultraviolet spectrophotometer) and the UV absorbing fraction was concentrated in vacuo to 22.16 g of pure R,S - α - hydroxymethyl - tyrosine methyl ether.

C) R,S-N-Acetyl- α -Hydroxymethyl-Tyrosine Methyl Ether

19.7 g of R,S - α - hydroxymethyl - tyrosine methyl ether (87.5 mmol) was suspended in 200 ml of dry pyridine, then 68 ml of acetic anhydride was added. After aging overnight at room temperature, the solution was concentrated in vacuo to dryness and azeotroped with 2x50 ml toluene. The residue was dissolved in 118 ml of methanol and 130 ml of aqueous 2.5 N NaOH solution and stirred at room temperature for 3.5 h. Acidification with 30 ml of conc. HCl followed by extraction with 4x200 ml of ethyl acetate, and then drying and concentration afforded 21 g of crude product. This was recrystallized from 75 ml of acetonitrile yielding 9.35 g of R,S - N - acetyl - α - hydroxymethyl - tyrosine methyl ether, mp 151—152°C dec.

D.) Optical Resolution of R,S-N-Acetyl- α -Hydroxymethyl-Tyrosine Methyl Ether

10 g of R,S - N - acetyl - α - hydroxymethyl - tyrosine methyl ether and 6.18 g of d-ephedrine were dissolved in 50 ml of methanol. The solution was concentrated to dryness in vacuo and then redissolved in 50 ml of warm acetonitrile. Crystallization afforded 7.34 g of the d-ephedrine salt of R - N - acetyl - α - hydroxymethyl - tyrosine methyl ether, mp 125—131°C (Crop A). Crop A was recrystallized from 40 ml of acetonitrile affording 4.78 g of Crop B, mp 130—134°C. The mother liquors from A and B were combined and concentrated, and the residues dissolved in 22.4 ml of 2.5 N NaOH and 50 ml of H_2O . The aqueous solutions were extracted with 2x75 ml ethyl acetate. The aqueous solutions were cooled and acidified with 5 ml of conc. HCl and the resultant solution extracted with 3x70 ml ethyl acetate. The dried organic solution was concentrated to 7.73 g (Crop C). Crop C and 4.7 g of l-ephedrine were dissolved in 50 ml of methanol and concentrated to 12.39 g (Crop D). This was recrystallized from 50 ml acetonitrile to yield 5.06 g of the l-ephedrine salt of S - N - acetyl - α - hydroxymethyl - tyrosine methyl ether (Crop E), mp 131.5—133.5°C dec. Crop E was recrystallized from 27 ml of acetonitrile to give Crop F, 4.72 g, mp 130.5—134.5°C dec. The mother liquors from Crop F and Crop E were combined and concentrated to 7.31 g (Crop G). Crop G was converted back to the free acid using the method used to obtain Crop C and there was obtained 3.0 g (Crop H). This was treated as was the initial R,S-material with 1.9 g of d-ephedrine. Recrystallization of the salt from 17 ml of acetonitrile afforded 2.4 g of Crop J, mp 127—130°C. Crop J was recrystallized to 2.06 g of Crop K, mp 130—134°C dec.

Combined Crops B and K (6.52 g) were recrystallized from 40 ml of acetonitrile affording 6.06 g of the d-ephedrine salt of R - N - acetyl - α - hydroxymethyl - tyrosine methyl ether (75.8% overall).

The free acid was regenerated in the same manner as that by which the combined mother liquors of Crops A and B were converted to Crop C and there was obtained 3.50 g of R - N - acetyl - α - hydroxymethyl - tyrosine methyl ether: $[\alpha]_D^{25} = +92^\circ$ (C, 1.35, 0.27 N NaOH).

E.) R- α -Hydroxymethyl-Tyrosine

3.3 g of R - N - acetyl - α - hydroxymethyl - tyrosine methyl ether was dissolved in 100 ml of conc. HCl and heated in a pressure tube at 130°C for 2 hr.

The solution was concentrated to dryness, the residue dissolved in 35 ml of H₂O, filtered and treated with 1 ml of pyridine. 2.11 g of pure R - α - hydroxymethyl - tyrosine (81%) crystallized out: $[\alpha]_D^{25} = 0.86^\circ$ (C, 1.15, 50% aqueous trifluoroacetic acid). The circular dichroism (CD) spectrum has the same sense as the CD of S - α - methyl - tyrosine.

F.) S- α -Fluoromethyl-Tyrosine

Following the procedure of Example 4, S - α - fluoromethyl - tyrosine was prepared from R - α - hydroxymethyl - tyrosine.

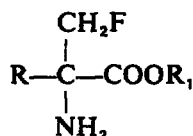
EXAMPLE 8

($\pm$)- α -Fluoromethylglutamic Acid

6.56 g of α -methylglutamic acid hemihydrate was photofluorinated in liquid HF solution by the general technique described in Journal of the American Chemical Society, 92, 7494 (1970) and 98, 5591 (1976). The substrate was dissolved in 120 ml of liquid Hf and irradiated with a 2500 W ultraviolet light source under stirring while fluoroxy-trifluoro-methane (CF₃OF) gas (3.0 ml as measured in liquid form at -78°C) was passed in the course of 80 min, under cooling in a dry-ice-acetone bath. After another 80-minute period with irradiation under similar conditions, an additional similar dose of CF₃OF was added in 3 hours, continuing with the stirring, cooling and irradiation. The mixture was kept overnight in the dry-ice-acetone bath, then it was further fluorinated (with 3 ml of CF₃OF, added in 5 hours with irradiation). Nitrogen gas was blown through the solution for removal of the solvent and the residue was evaporated with 2.5 N aq. HCl (2x) in vacuo. The residue was dissolved in 40 ml of water. To 10 ml of this solution 10 ml of conc. HCl was added and the mixture was refluxed for about 68 hours. After treatment with Darco G-60, the filtrate was evaporated in vacuo and the residue refluxed with 30 ml of conc. HCl for another 68-hour period. After treatment with Darco, the solution was evaporated to dryness, dissolved in 10 ml of conc. HCl and heated in a sealed glass tube for 24 hours in an oil-bath kept at 130—135°. Evaporation *in vacuo* to dryness gave a residue which was dissolved in H₂O and subjected to elution chromatography on a cation-exchange resin column, made of 360 ml of Dowex AG50-X12 (mesh 200/400). Eluants: 2.6 l of H₂O, followed by 0.1 N aq. HCl (1.5 l), then by 0.15 N aq. HCl. UV absorption of the effluent was monitored by a recording UV at 206 nm. 15-ml fractions of effluent were collected and 20 fractions, corresponding to the first ultraviolet absorbing peak, were combined and evaporated in vacuo to dryness, to give α -fluoromethyl-glutamic acid hydrochloride. For liberation of the amino acid, this was dissolved in isopropanol and filtered, and then propylene oxide was added. α -Fluoromethyl-glutamic acid. 0.7 H₂O crystallized out of the solution. This compound is a time-dependent inhibitor of glutamic acid decarboxylase.

WHAT WE CLAIM IS:—

1. A compound having the formula

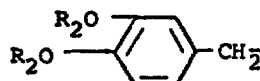


wherein R is a substituted C₁—C₄ alkyl group and R₁ is H or C₁—C₁₈ alkyl.

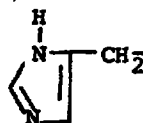
2. Pharmaceutically acceptable acid addition salts of a compound as claimed in Claim 1.

3. A compound as claimed in Claim 1 having the S-isomer configuration.

4. A compound as claimed in Claim 1 in which the substituted alkyl group is



wherein R₂ is H or C₂—C₆ alkanoyl,





The solution was concentrated to dryness, the residue dissolved in 35 ml of H₂O, filtered and treated with 1 ml of pyridine. 2.11 g of pure R - α - hydroxymethyl - tyrosine (81%) crystallized out: $[\alpha]_D^{25} = 0.86^\circ$ (C, 1.15, 50% aqueous trifluoroacetic acid). The circular dichroism (CD) spectrum has the same sense as the CD of S - α - methyl - tyrosine.

F.) S- α -Fluoromethyl-Tyrosine

Following the procedure of Example 4, S - α - fluoromethyl - tyrosine was prepared from R - α - hydroxymethyl - tyrosine.

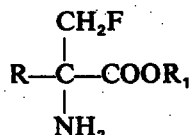
EXAMPLE 8

($\pm$)- α -Fluoromethylglutamic Acid

6.56 g of α -methylglutamic acid hemihydrate was photofluorinated in liquid HF solution by the general technique described in Journal of the American Chemical Society, 92, 7494 (1970) and 98, 5591 (1976). The substrate was dissolved in 120 ml of liquid Hf and irradiated with a 2500 W ultraviolet light source under stirring while fluoroxy-trifluoro-methane (CF₃OF) gas (3.0 ml as measured in liquid form at -78°C) was passed in the course of 80 min, under cooling in a dry-ice-acetone bath. After another 80-minute period with irradiation under similar conditions, an additional similar dose of CF₃OF was added in 3 hours, continuing with the stirring, cooling and irradiation. The mixture was kept overnight in the dry-ice-acetone bath, then it was further fluorinated (with 3 ml of CF₃OF, added in 5 hours with irradiation). Nitrogen gas was blown through the solution for removal of the solvent and the residue was evaporated with 2.5 N aq. HCl (2x) in vacuo. The residue was dissolved in 40 ml of water. To 10 ml of this solution 10 ml of conc. HCl was added and the mixture was refluxed for about 68 hours. After treatment with Darco G-60, the filtrate was evaporated in vacuo and the residue refluxed with 30 ml of conc. HCl for another 68-hour period. After treatment with Darco, the solution was evaporated to dryness, dissolved in 10 ml of conc. HCl and heated in a sealed glass tube for 24 hours in an oil-bath kept at 130—135°. Evaporation *in vacuo* to dryness gave a residue which was dissolved in H₂O and subjected to elution chromatography on a cation-exchange resin column, made of 360 ml of Dowex AG50-X12 (mesh 200/400). Eluants: 2.6 l of H₂O, followed by 0.1 N aq. HCl (1.5 l), then by 0.15 N aq. HCl. UV absorption of the effluent was monitored by a recording UV at 206 nm. 15-ml fractions of effluent were collected and 20 fractions, corresponding to the first ultraviolet absorbing peak, were combined and evaporated in vacuo to dryness, to give α -fluoromethyl-glutamic acid hydrochloride. For liberation of the amino acid, this was dissolved in isopropanol and filtered, and then propylene oxide was added. α -Fluoromethyl-glutamic acid. 0.7 H₂O crystallized out of the solution. This compound is a time-dependent inhibitor of glutamic acid decarboxylase.

WHAT WE CLAIM IS:—

1. A compound having the formula

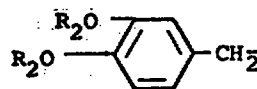


wherein R is a substituted C₁—C₄ alkyl group and R₁ is H or C₁—C₁₈ alkyl.

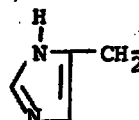
2. Pharmaceutically acceptable acid addition salts of a compound as claimed in Claim 1.

3. A compound as claimed in Claim 1 having the S-isomer configuration.

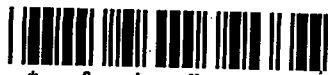
4. A compound as claimed in Claim 1 in which the substituted alkyl group is



wherein R₂ is H or C₂—C₈ alkanoyl,



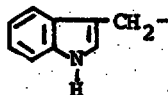
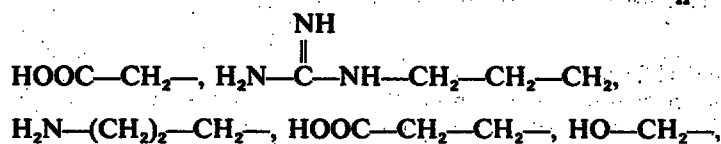
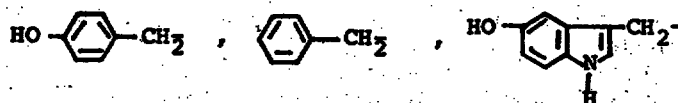
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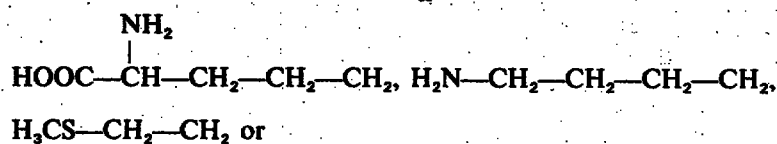
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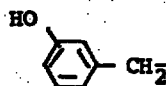
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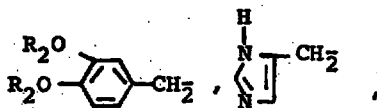


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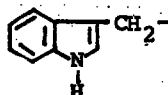
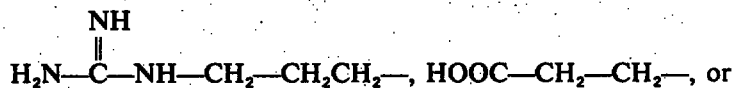
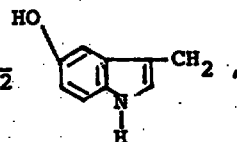
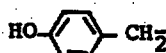
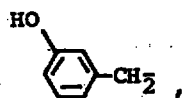
and R_1 is H.

5. A compound as claimed in Claim 4 in which the substituted alkyl group is

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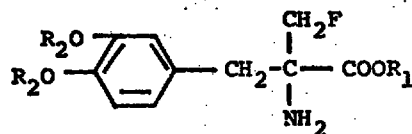
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6. A compound having the formula

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where R_1 and R_2 are as defined in Claims 1 and 4 respectively.7. A compound as claimed in Claim 6 in which R_2 is hydrogen and R_1 is H or ethyl.

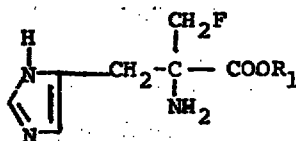
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8. A compound as claimed in Claim 7 in which R_1 is hydrogen.

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9. A compound as claimed in Claim 8 having the S-isomer configuration.

10. A compound as claimed in Claim 1 having the formula



11. A compound as claimed in Claim 10 having the formula

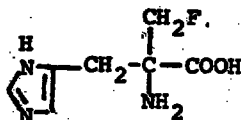


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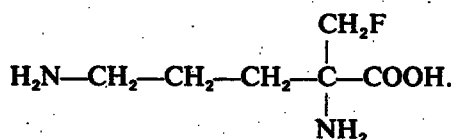
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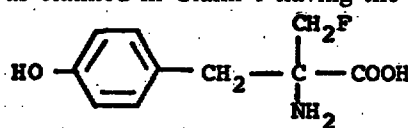


12. A compound as claimed in Claim 11 having the S-isomer configuration.
13. A compound as claimed in Claim 1 having the formula



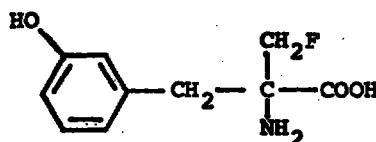
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14. A compound as claimed in Claim 13 having the S-isomer configuration.
15. A compound as claimed in Claim 1 having the formula



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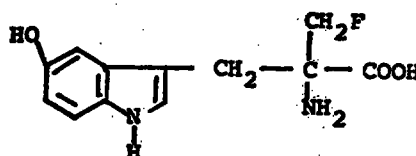
16. A compound as claimed in Claim 15 having the S-isomer configuration.
17. A compound as claimed in Claim 1 having the formula



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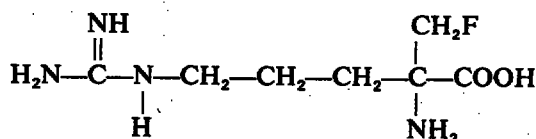
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18. A compound as claimed in Claim 17 having the S-isomer configuration.
19. A compound as claimed in Claim 1 having the formula



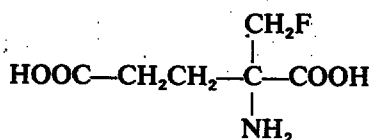
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20. A compound as claimed in Claim 19 having the S-isomer configuration.
21. A compound as claimed in Claim 1 having the formula



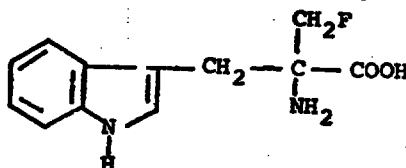
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22. A compound as claimed in Claim 21 having the S-isomer configuration.
23. A compound as claimed in Claim 1 having the formula:



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24. A compound as claimed in Claim 23 having the S-isomer configuration.
25. A compound as claimed in Claim 1 having the formula:



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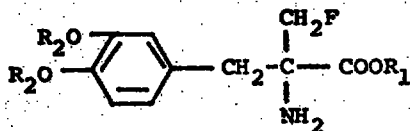
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26. A compound as claimed in Claim 25 having the S-isomer configuration.

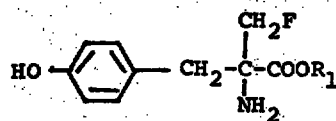
27. A pharmaceutical composition containing a compound of Claim 1 or a pharmaceutically acceptable acid-addition salt thereof, together with a pharmaceutically acceptable diluent.

28. A composition as claimed in Claim 27 in which the said compound has the formula



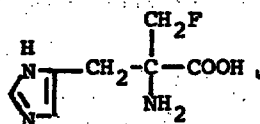
where R_1 is H or C_1-C_6 alkyl and R_2 is H or C_2-C_6 alkanoyl.

29. A composition as claimed in Claim 27 in which the said compound has the formula

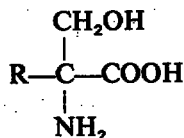


where R_1 is H or C_1-C_6 alkyl.

30. A composition as claimed in Claim 27 in which the said compound has the formula

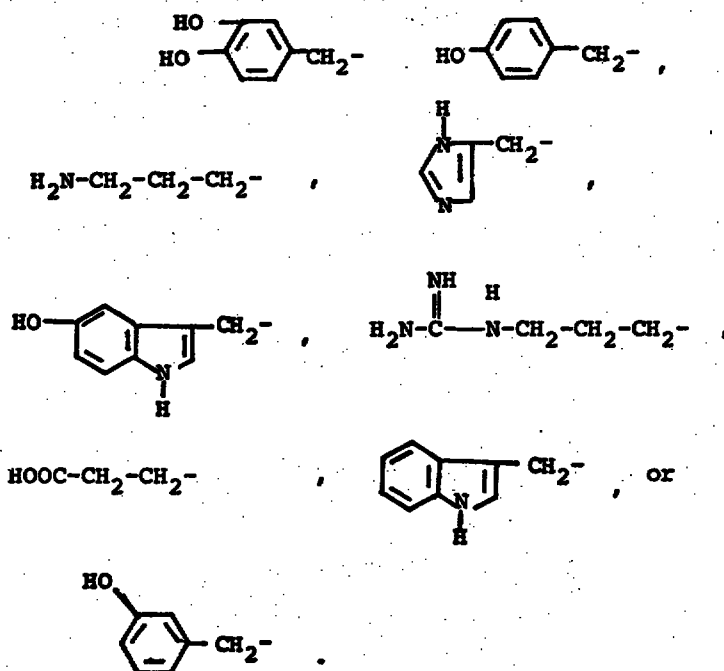


31. A process for preparing a compound of Claim 1 in which R_1 is H which comprises fluorodehydroxylation of a compound having the formula



by treatment with sulfur tetrafluoride in hydrofluoric acid.

32. A process as claimed in Claim 31 in which R is



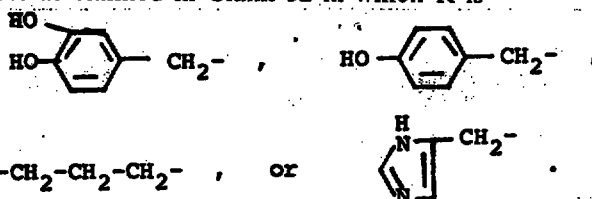


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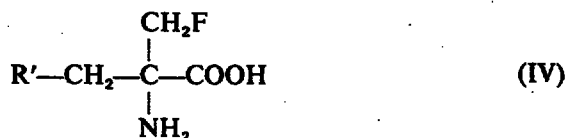
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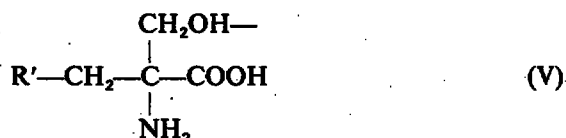
33. A process as claimed in Claim 32 in which R is



34. A process for preparing compounds having the formula

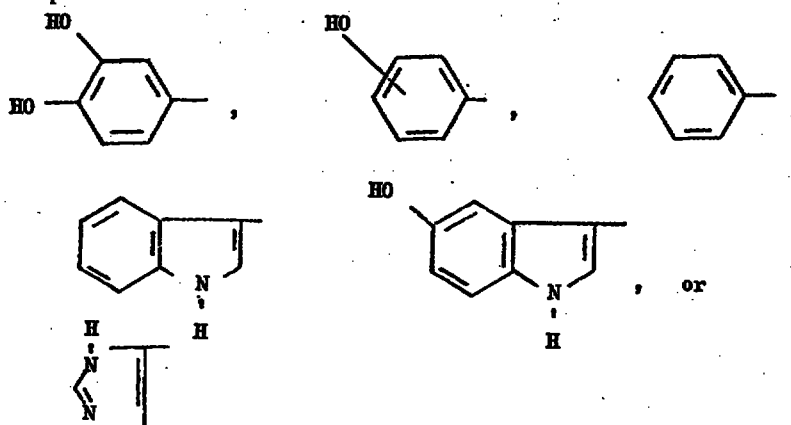


where R' is an aryl group, comprising reacting a compound having the formula



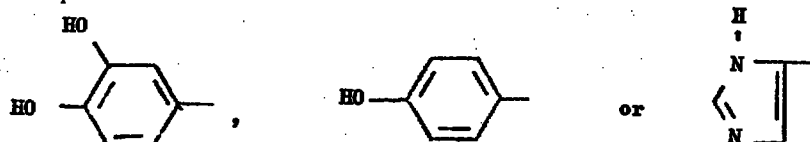
with SF₄ in liquid HF at temperatures ranging from -80°C to 20°C, in which BF₃ or AlCl₃ is present in the reaction system.

35. A process as claimed in Claim 34 in which R' is



and the added compound is BF₃.

36. A process as claimed in Claim 35 in which R' is



37. A process as claimed in Claim 35 in which the Formula IV and V compounds have the S-isomer configuration.

38. A compound as claimed in Claim 1, when prepared by a process substantially as hereinbefore described in any one of the foregoing Examples.

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