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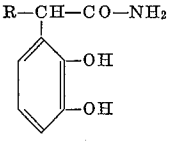
# 2,3-DIHYDROXYPHENYLALKANAMIDES

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No Drawing. Continuation of application Ser. No. 370,056, May 25, 1964, which is a continuation-in-part of application Ser. No. 251,019, Jan. 14, 1963. This application Feb. 13, 1967, Ser. No. 615,840
Claims priority, application Sweden, Jan. 17, 1962, 467/62; Jan. 23, 1964, 798/64
U.S. Cl. 260—559 7 Claims
Int. Cl. C07c *103/26*

## ABSTRACT OF THE DISCLOSURE

The amides have the formula:



the group R being an alkyl or alkoxy group containing at most 4 carbon atoms. The amides inhibit the enzymatic hydroxylation of aromatic L-amino acids. They are used in the treatment of mentally deficient patients and diseases of circulation, especially hypertension. They may be prepared by reacting the corresponding free acids or an amide forming derivative thereof with ammonia; the nuclear hydroxy groups may be protected during such reaction.

## Cross-reference to related applications

This application is a continuation of our application No. 370,056 filed May 25, 1964 which is itself a continuation-in-part of our application No. 251,019 filed Jan. 14, 1963, both of which are now abandoned.

This invention relates to physiologically active amides and their preparation.

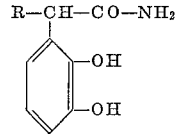
It is known that aromatic L-amino acids are converted by enzymatic hydroxylation into corresponding mono- and di-hydroxyamino acids which in their turn are precursors in the biosynthesis of catecholamines. Thus, the essential amino acid L-phenylalanine is a biological precursor of adrenalin (epinephrine) and it is assumed that the metabolic conversion of one to the other includes, as a first step, hydroxylation to L-tyrosine which is then converted into 3,4-dihydroxyphenylalanine, commonly known as "dopa." An analogous hydroxylation takes place when tryptophane is metabolized to L-5-hydroxytryptophane, which is, in its turn, a precursor for 5-hydroxytryptamine. Since the steps tyrosine→dopa and trytophane→5-hydroxytryptophane are rate determining in the biosynthesis of the important biological amines noradrenalin (norepinephrine) and 5-hydroxytryptamine, inhibition of these enzymatic hydroxylations produces important physiological effects.

In mammals, inhibition of these hydroxylations causes a lowering of the concentration of catecholamines and hydroxytryptamine in various tissues such as the heart and brain. Especially in the treatment of mentally deficient patients, it is of great importance to be able in this way to control and regulate the concentration of catecholamines and hydroxytryptamine. Indirectly, this is corroborated by the fact that the alkaloid reserpine, which causes a lowering of these concentrations, is used therapeutically. Reserpine, however, produces this effect by another mechanism.



A lowering of the concentration of catecholamines is also of great importance in the treatment of diseases of the circulation, especially hypertension.

It has now been found that the new compounds which have the general formula:



I

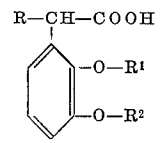
in which R denotes an alkyl or alkoxy group containing not more than four carbon atoms, inhibit the enzymatic hydroxylation of aromatic L-amino acids. This inhibiting effect with its important biochemical consequences is obtained with small doses of the compounds which are, furthermore, of very low toxicity. For these reasons the compounds of Formula I find use in medicine.

The effect of the new compounds may be demonstrated by tests in mice. In these animals the monoamine-oxidase-inhibiting compound nialamide, [N-benzyl-β-(isonicotinoylhydrazino)-propionamide], causes what has become known as model psychoses. The development of this syndrome is effectively blocked by the administration of the new compounds.

The ability of these compounds to block the enzymatic hydroxylation of aromatic L-amino acids may also be demonstrated in another way. If they are administered to reserpine-treated mice, a characteristic relaxing effect is obtained, the crooked position disappears, the animals lose the righting reflex and fall asleep. This very interesting effect is dose-dependent and the depth and duration of the hypnosis depends on the time-interval between the injections of reserpine and the compound tested. That this effect is really a consequence of an inhibition or blocking of the enzymatic hydroxylation is evident from the fact that if dopa is injected intraperitoneally the animals wake up rapidly. After this injection they show, furthermore, the same symptoms of adrenergic superactivity as injected control animals.

The new compounds inhibit tyrosinehydroxylase but do not inhibit, in vitro or in vivo, the enzyme catechol-O-methyltransferase (COMT). In this respect they differ from the corresponding 3,4-dihydroxy compounds, which inhibit both enzymes. As COMT is an enzyme which inactivates catecholamine, whereas tyrosinehydroxylase controls the rate limiting step in the biosynthesis of catecholamines, it is evidently of value to separate the COMT- and tyrosinehydroxylase-inhibiting properties of the 3,4-dihydroxyphenylacetamides. In certain clinical situations where a blockade of the biosynthesis of catecholamines is desired, the absence of any COMT-inhibiting potency is mandatory, e.g. in cases of phaeochromocytoma. The same is true for certain mental disorders where a clearcut inhibition of the biosynthesis of catecholamines is highly desirable, in order to determine the importance of the two enzymes in the central nervous system.

The new amides of the general Formula I may be prepared according to methods known per se by reacting with ammonia an acid of the general formula:



II

where R is as hereinbefore defined and $R^1$ and $R^2$ denote hydrogen or protecting groups, or an amide-forming derivative, such as an acid halide, anhydride (or half-anhydride with another acid), esters or lactone (formed

by internal reaction of the carboxyl groups with the 2-hydroxy group) of such an acid. The protecting groups, when present, are removed during or after the conversion. Suitable protecting groups are lower alkyl groups, i.e. alkyl groups containing not more than five carbon atoms, aralkyl groups, such as benzyl groups, removed by hydrogenolysis, and acyl groups, e.g. acetyl or benzoyl, removed by hydrolysis. The conversion is conveniently carried out by reacting an acid of Formula II as such or, more preferably, as a lactone or acid chloride, with ammonia. When an acid chloride is used $R^1$ and $R^2$ are preferably both benzyl groups which are subsequently removed by catalytic hydrogenation.

The following examples illustrate the invention.

## EXAMPLE 1

A mixture of 230 ml. of hydroiodic acid ($d$=1.7) and 18 g. of red phosphorus was boiled for 30 minutes. 54.1 g. of α-ethyl-2,3-dimethoxyphenylacetic acid (II, R=$C_2H_5$, $R^1$=$R^2$=$CH_3$) were added and the mixture was boiled with reflux during 3 hours. It was then evaporated to dryness 3 times with 10 ml. of water added between each evaporation. The residue was dissolved in ethyl acetate, the phosphorus was removed and the solution washed first with a solution of 57 g. of sodiumpyrosulphite in 156 ml. of water and then with 150 ml. of water. After drying and evaporation the resulting lactone of α-ethyl-2,3-dihydroxyphenylacetic acid was distilled, yield 38.6 g., B.P. 114–117° C./0.05 mm. Hg.

18 g. of the compound thus obtained, 20 ml. of toluene and 200 ml. of liquid ammonia were mixed in an autoclave and heated to 100° C. After 2 hours the reaction mixture was poured into water and acidified with conc. HCl at below 20° C. The resulting α-ethyl 2,3-dihydroxyphenylacetamide was recrystallized from water, yield 15 g., M.P. 124–125° C.

The starting material, α-ethyl-2,3-dimethoxyphenylacetic acid, was prepared from 2,3-dimethoxyphenylacetonitrile by ethylation with ethyl iodide and sodium ethoxide and hydrolysis of the intermediate α-ethyl-2,3-dimethoxyphenylacetonitrile.

In an analagous way the following compounds were prepared: α-methyl-2,3-dihydroxyphenylacetamide, M.P. 150–156° C.; α-n-propyl-2,3-dihydroxyphenylacetamide, M.P. 113–114° C.; α-isopropyl-2,3-dihydroxyphenylacetamide, M.P. 117° C.; and α-isobutyl-2,3-dihydroxyphenylacetamide, an oil, identified by its IR-absorption.

## EXAMPLE 2

31.0 g. of 2,3-dibenzyloxyphenylacetic acid were dissolved in 150 ml. of $CHCl_3$ and 60 ml. of thionylchloride were added. After boiling with reflux for 2 hours, the solvent was evaporated and the resulting oily acid chloride used directly for amidation.

34.8 g. of the acid chloride were dissolved in 70 ml. of anhydrous dioxane and the solution was added drop by drop with vigorous stirring to a mixture of 70 ml. of dioxane and 70 ml. of 25% $NH_4OH$. The resulting mixture was kept for 1 hour at room temperature with stirring after which 200 ml. of ice and water were added and the stirring continued for 1 hour. The resulting crude product was collected, washed, dried and dissolved in methanol. The methanol solution was treated with active carbon, evaporated to dryness and the remainder recrystallized from ethyl acetate-petroleum ether. 20.8 g. of 2,3-dibenzyloxyphenylacetamide, M.P. 178–180° C., were obtained, which were dissolved in 550 ml. of methanol and hydrogenated (using 2 g. of Pd/C). After absorption of 2850 ml. of hydrogen, the reaction mixture was filtered and evaporated and the residue recrystallized from ethyl acetate-petroleum ether. A quantitative yield of 2,3-dihydroxyphenylacetamide was obtained, M.P. 169–170° C.

The starting material, 2,3-dibenzyloxyphenylacetic acid was prepared from 2,3-dibenzyloxybenzylalcohol which was converted into 2,3-dibenzyloxybenzylchloride by treatment with thionyl chloride. 2,3-dibenzyl-oxybenzylchloride was treated with sodium cyanide in methyl ethyl ketone to give 2,3-dibenzyloxyphenylacetonitrile, which was then hydrolyzed to 2,3-dibenzyloxyphenylacetic acid with sodium hydroxide.

In an analogous way, α-n-propyl-2,3-dihydroxyphenylacetamide, M.P. 114° C., was obtained.

## EXAMPLE 3

25 g. of α-methoxy-2,3-dibenzyloxyphenylacetic acid were dissolved in 70 ml. of thionyl chloride at 0° C. The reaction was started by the addition of 5 drops of pyridine. The mixture was kept at 0° C. for 1 hour, then at 20° C. for 2 hours, and then evaporated in vacuo at a temperature below 25° C. The resulting acid chloride was dissolved in 75 ml. of dioxane and added with cooling to a mixture of 160 ml. of dioxane and 80 ml. of concentrated $NH_4OH$. After 1 hour, much water was added, the resulting amide was collected and recrystallized from trichloroethylene petroleum ether M.P. 85° C.

5.0 g. of the amide thus obtained were dissolved in 100 ml. of ethanol and hydrogenated (0.5 g. of Pd/C). After the absorption of 650 ml. of hydrogen, the reaction mixture was filtered and evaporated. α-Methoxy-2,3-dihydroxyphenylacetamide was obtained as a viscous oil which crystallized slowly in a refrigerator.

The starting material, α-methoxy-2,3-dibenzyloxyphenylacetic acid was obtained by treating 2,3-dibenzyloxybenzaldehyde with bromoform, methanol and potassium hydroxide in a dioxane solution.

In an analogous way, α-ethoxy-2,3-dihydroxyphenylacetamide, M.P. 120° C., and α-n-propoxy-2,3-dihydroxyphenylacetamide, M.P. 84° C., were obtained.

The physiological effect of the compounds of Formula I may be shown by the following tests.

(a) Blocking of the nialamide syndrome.—By the intraperitoneal administration in mice of 0.5 g./kg. nialamide a model psychosis is developed in control animals. If a compound of the invention is also administered the development of the syndrome is blocked. In addition, analysis of the brain of mice so treated demonstrates that the accumulation of 5-hydroxytryptamine, which otherwise follows treatment with a mono-amine oxidase inhibitor, is also blocked. This is shown by the following Table I.

TABLE I

[Mice injected intraperitoneally with 0.5 g./kg. of nialamide. 5-hydroxytryptamine (5-HT) determined in brain after 2 hours]

| Compound of formula I; R= | Dose administered (g./kg.) | 5-HT in brain (ug./g.) |
|---|---|---|
| Control | 0 | 1.28 |
| $C_2H_5$—O | 0.3 | 0.97 |
| $C_2H_5$—O | 0.5 | 0.66 |
| $C_2H_5$ | 0.5 | 0.63 |

(b) Modification of the reserpine effect.—5 mg./kg. of reserpine were injected intraperitoneally in mice. After 22 hours a compound of the invention was administered. At this the animals lost the characteristic crooked position, relaxed, the righting reflex disappeared and the animals fell asleep. The duration of the hypnosis depended on the dose of compound administered.

When L-dopa was then injected intraperitoneally, the animals were rapidly awakened and showed the known symptoms of adrenergic superactivity (aggression, increased motility, piloerection, exophthalamus).

These tests demonstrate that the hydroxylase-blocking effect of the new substances is responsible for the potentiation of the reserpine effect. The activity of a representative selection of compounds according to the invention is shown in the following Table II.

TABLE II

| Compound of formula I; R= | Dose, g./kg. | Effect | Duration, min. |
|---|---|---|---|
| $CH_3$ | 1.0 | Weak | 20 |
| $C_2H_5$ | 0.5 | Moderate | 40 |
| $C_3H_7$ | 0.5 | do | 40 |
| Iso-$C_3H_7$ | 0.5 | Strong | 40 |
| Iso-$C_4H_9$ | 0.5 | Weak | |
| $CH_3$·O | 0.5 | Moderate | 30 |
| $C_2H_5$·O | 0.25 | Strong | 60 |
| $C_2H_5$·O | 0.5 | do | 120 |
| n-$C_3H_7$·O | 0.5 | do | 60 |

(c) Lowering of the concentration of catecholamines and 5-hydroxy-tryptamine in mouse-brain.—Mice were injected intraperitoneally with varying doses of the new compounds and killed after 90 minutes. The concentration of catecholamines and 5-HT in the brains was then determined. It should be noted that even small reductions of the concentrations of these important biological amines by inhibition of biosynthesis have far-reaching consequences. The results are shown in the following Table III.

TABLE III

| Injected compound of formula I, R= | Dose, g./kg. | Concentration in the brain of (μg./g.) of— | | |
|---|---|---|---|---|
| | | Dopamine | Noradrenalin | 5-HT |
| Control | 0 | 0.98 | 0.50 | 0.45 |
| $C_2H_5$ | 1.0 | 0.92 | 0.35 | 0.35 |
| $C_2H_5$·O | 0.5 | 0.92 | 0.36 | 0 32 |
| $C_2H_5$·O | 1.0 | 0.69 | 0.37 | 0.28 |

(d) Absence of inhibiting effect on catechol-O-methyl-transferase.—Mice were injected intraperitoneally with reserpine (5 mg./kg.) and treated with nialamide (0.1 g./kg. intraperitoneally) 22 hours later. After a further 30 minutes, 0.5 g./kg. of the 2,3-dopacetamide was administered intraperitoneally. 30 minutes later L-dopa (7.5 mg./kg.) was injected intraperitoneally and the animals were killed after one hour. The concentration of 3-methoxytyramine in the brain was then determined. In all cases the values obtained (0.4–0.5 μg./kg.) were, within experimental error, the same as in untreated control animals. This demonstrates that the compounds of the invention do not possess any inhibiting activity on catechol-O-methyl-transferase, which would have resulted in a lowering of the concentration of 3-methoxytyramine. Also, there were no signs during the tests of adrenergic superactivity in the treated animals.

Experiments have also demonstrated that no inhibiting activity exists in vitro.

The invention includes within its scope pharmaceutical compositions comprising, in association with a pharmaceutical carrier compatible therewith, which may be either a solid material or a liquid, an amide of Formula I. These pharmaceutical compositions can take the form of tablets, powders, capsules, and similar dosage forms for oral administration or for use as a suppository. The active ingredient can also be administered orally as an ingredient in an elixir, a suspension or an emulsion. For the injectable compositions, sterile liquids, preferably distilled water, are used as solvents. The ratio of the amount of active substance to carrier varies according to the nature of the composition, but is, as a rule, within the range of from 1:1 to 1:10,000.

When administered orally the compounds of the invention may be given in widely varying dosages from, for example, 20 mg./day to 2 g./day, but dosages of 200–1000 mg./day will ordinarily be given, amounts between 300 and 600 mg./day being usually preferred.

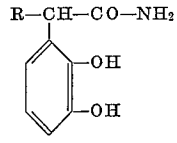
The following is an example of a pharmaceutical composition in accordance with the invention.

EXAMPLE 4

α-Ethoxy-2,3-dihydroxyphenylacetamide (50 g.), lactose (100 g.), calcium citrate (50 g.), and starch (50 g.) are mixed together and granulated using a 10% aqueous gelatin solution. The granules are passed through a 20-mesh sieve, mixed with magnesium stearate (1.5 g.) and talc (5 g.), and then tableted using a 9 mm. punch.

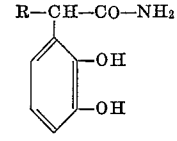
We claim:

1. An amide of the formula:




in which R is a member selected from the class consisting of alkyl and alkoxy groups of at most four carbon atoms.

2. α-Ethyl-2,3-dihydroxyphenylacetamide.
3. α-n-Propyl-2,3-dihydroxyphenylacetamide.
4. α-Isopropyl-2,3-dihydroxyphenylacetamide.
5. α-Ethoxy-2,3-dihydroxyphenylacetamide.
6. α-n-Propoxy-2,3-dihydroxyphenylacetamide.
7. An amide of the formula:




wherein R is selected from the group consisting of methyl, ethyl, n-propyl, and isopropyl.

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