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METHOD OF LOWERING BLOOD CHOLESTEROL LEVEL

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This invention is concerned with new therapeutic compositions having unusual activity in lowering blood cholesterol levels. More particularly, the invention relates to certain lower hydroxyamides which have hypocholesteremic properties and to the formulation of these amides as therapeutically useful compositions.

The active hypocholesteremic agents in accordance with the present invention can be identified as hydroxyamides of the following formula:

$$HO-(CH_2)_n-\overset{\overset{O}{\|}}{C}-\overset{\overset{H}{|}}{N}-R$$

wherein n is an integer from 1–4 and R is a lower alkyl group in the range C_4–C_5 or a β-phenethyl group. Thus the acyl portion of these agents can be characterized by their having but one hydroxyl group, present at the terminal carbon, and a straight chain interposed between the carbonyl group and the hydroxyl group.

More specifically, the present invention and the foregoing formula embraces the active compounds typified in the table below which characterizes their absence of toxicity and hypocholesteremic properties.

TABLE I.—HYPOCHOLESTEREMIC EFFECT

n	R	LD_{min} [a] s.c. in Mice, mg./kg.	Percent reduction in cholesterol (72 hrs.) [b]
1	$C_6H_5CH_2CH_2$—	750	45
2	$C_6H_5CH_2CH_2$—	750	35
4	$C_6H_5CH_2CH_2$—	1,000	34
3	n-C_5H_{11}—	750	34
4	n-C_5H_{11}—	1,000	49

[a] The LD_{min} is the minimum dosage which is lethal to mice upon subcutaneous injection expressed in mg./kg.

[b] The hypocholesteremic effect was evaluated in guinea pigs given 30 mg./kg. orally of the test compound at the beginning of the experiment, 24 hours later, and finally, 48 hours later. Serum cholesterol levels were established at the initiation of the experiment and at 72 hours thereafter, and the percent reduction from the cholesterol level at the initiation of the experiment noted.

In particular, the selected composition of this invention has N-β-phenethyl glycolamide as its essential active ingredient.

Further criteria characterizing the novel properties of the aforesaid compounds is that the hypocholesteremic effect be obtained upon oral administration and that the essential active ingredients be substantially without toxicity and that they exert little or no effect in other pharmacological areas at dosage levels consistent with good hypocholesteremic activity.

The compounds of this invention are active, however, when administered orally, rectally, or parenterally.

The active ingredients are obtained as distillable oils or low melting solids and are somewhat soluble in water, but for therapeutic use, they are preferably incorporated in suitable pharmaceutical carriers and in use, the compounds are employed in the recommended dosage of 100–1200 mg./day, preferably in doses of 150–300 mg., two or four times a day. Thus, in preparing tablets, sustained-release capsules, capsules, elixers, suppositories, or other dosage forms with pharmaceutical carriers, the formulation should preferably contain 50–500 mg. of active drug per dosage unit.

As more fully illustrative of the pharmacological standards of active ingredients typifying this invention, there is described below a variety of tests with N-(β-phenethyl) glycolamide in greater detail, to indicate its relative absence of other pharmacological effects and its substantial absence of toxicity.

Acute toxicity data are shown in Table II.

TABLE II [a].—ACUTE TOXICITY OF N-(β-PHENETHYL)GLYCOLAMIDE

Species	Route	LD_{50} (mg./kg.)
Mouse	Oral	1,500
Guinea pig	Oral	1,350
Rat	Oral	2,450

[a] Deaths were recorded for a week after a single administration.

The drug was inactive in the following pharmacological tests (30 and 60 mg./kg. orally): anticonvulsant, antitremorine, muscle relaxant, bronchodilator. No central nervous excitatory or depressant activity was observed up to 100 mg./kg. S.C. and 60 mg./kg. orally. It had no local anesthetic action on the guinea pig eye at 20 mg./ml.

Injected intravenously to anesthetized dogs at 5 mg./kg., N-(β-phenethyl)glycolamide was without significant effect on blood pressure, heart rate, or the rate of respiration. It did not modify the response to subsequent injections of adrenaline, acetylcholine or histamine.

Addition of the drug to isolated guinea pig ileum and rat uterus at 40 μg. per ml. had no effect on the contraction or on the response of the tissues to histamine and acetylcholine.

The bromsulfalein test and prothrombin time were normal after 3 days' administration.

The compound had no analgesic effect on testing as high as 250 mg./kg. subcutaneously, but at these substantially higher doses and upon S.C. injection, it had anticonvulsant activity.

In general, at doses consistent with good hypocholesteremic effect, no other pharmacological effects were noted.

Upon oral administration to rats for 6 weeks, at 30, 150 and 600 mg./kg. (10 rats per group) there were no deaths at 30 and 150 mg./kg. levels whereas at the 600 mg./kg. group, one death after 16 days on test was noted. No observed changes due to drug treatment were noted during this period of feeding the drug and no significant differences in body weight over that obtained with the controls. The hematological studies at the end of the test showed no significant difference in red and white counts or in hemoglobin levels over that noted with the controls. Blood chemistry studies indicated no significant change in blood glucose, urea nitrogen, or serum protein levels when compared to the controls. Histological studies on the organs after completion of the chronic toxicity test showed no pathological findings at any of the dosage levels.

Compounds which have this desirable activity can be prepared in various ways. Thus, for the compounds where n=1, the desired route is by reaction of the appropriate amine, RNH_2, with ethyl glycolate as detailed by Shapiro et al., J. Am. Chem. Soc., 81, 6322 (1959). Alternative procedures involve condensation under reflux of the amine with glycolic acid in xylene with removal of formed water by means of a Dean-Stark trap.

The corresponding hydroxyamides where n=2 are obtainable preferably by the reaction of the amine with

β-propiolactone following the procedure of Gresham et al., J. Am. Chem. Soc., 73, 3168 (1951).

The corresponding hydroxyamides where $n=3$ and 4, are obtained by condensation of the amine with butyrolactone and valerolactone, respectively.

The following examples reflect the mode of preparation of the essential active ingredients of the present invention as well as the preparation of typical administerable formulations of said active ingredients but it is to be understood that these examples are given by way of illustration and not by limitation.

Example 1.—N-(β-phenethyl)glycolamide

A mixture of 126 g. (1.03 mole) of β-phenethylamine and 96 g. (0.924 mole) of ethyl glycolate was heated under reflux for 22 hours. A Dean-Stark trap was interposed to remove the formed ethanol (theory 54 ml.). When 48 ml. of ethanol had been collected and the internal temperature was 145°, the residue was cooled and 270 ml. ethyl acetate added. On standing, the product (107.5 g.) separated, M.P. 61–62°; addition of hexane to the mother liquor yielded an additional 27 g., M.P. 65–70°. The combined yield (134.5 g.) was stirred with 180 ml. water and the precipitate washed with ice water to give 96 g., M.P. 77–78° (58%), which on recrystallization (acetone-hexane) gave 89.7 g. of product, M.P. 77–78°.

In a similar manner, employing the appropriate amine, there is obtained N-(n-amyl)glycolamide, N-(i-amyl) glycolamide, N-(n-butyl)glycolamide.

Example 2.—N-(n-amyl)-β-hydroxypropionamide

To a solution of 7.2 g. (0.1 mole) of propiolactone in 50 ml. of water cooled to 0–5°, there was added 8.7 g. (0.1 mole) n-amylamine over 15 minutes with stirring. Water was removed by vacuum distillation. The residue was stirred with hexane, the hexane removed and the residue (11.9 g.) distilled to give 6.2 g., B.P. 130–132° (0.5 mm.), n_D^{20} 1.4714.

Analysis.—Calcd. for $C_8H_{17}NO_2$: C, 60.3; H, 10.8; N, 8.8. Found: C, 60.1; H, 10.4; N, 9.1.

In a similar manner using the appropriate amine, there is obtained N-(i-amyl)-β-hydroxypropionamide, and N-(i-butyl)-β-hydroxypropionamide.

Example 3.—N-(n-amyl)-δ-hydroxyvaleramide

A mixture of 7.0 g. (0.07 mole) of δ-valerolactone and 6.1 g. (0.07 mole) of n-amylamine was heated for 18 hours at 60–70° and then for an additional 20 hours at 90–100°. When cool, the formed solid was recrystallized (ethyl acetate-pentane-ether) to give 7.3 g. (56%) of product, M.P. 38°.

Analysis.—Calcd. for $C_{10}H_{21}NO_2$: C, 64.1; H, 11.3; N, 7.5. Found: C, 63.9; H, 11.1; N, 7.1.

In a similar manner using n-butylamine, there is obtained N-(n-butyl)-δ-hydroxvaleramide.

Example 4.—N-(n-amyl)-γ-hydroxybutyramide

A mixture of 8.6 g. (0.1 mole) of γ-butyrolactone and 8.7 g. (0.1 mole) of n-amylamine was maintained at 20° over 9 days. Upon addition of pentane and ethyl acetate and cooling to 5° there was obtained 13.0 g. of product, M.P. 34–36°, which on recrystallization (acetone-hexane) gave 9.2 g. (53%) of pure product, M.P. 35°.

Analysis.—Calcd. for $C_9H_{19}NO_2$: C, 62.4; H, 11.1; N, 8.1. Found: C, 62.4; H, 11.4; N, 8.0.

In a similar manner using the appropriate amine, there is obtained N-(β-phenethyl)-γ-hydroxybutyramide, N-(i-amyl)-γ-hydroxybutyramide, N - (i-butyl) - γ - hydroxybutyramide, N-(n-butyl)-γ-hydroxybutyramide.

Example 5.—N-(β-phenethyl)-δ-hydroxyvaleramide

A mixture of 7.0 g. (0.7 mole) of δ-valerolactone and 8.5 g. (0.7 mole) of β-phenethylamine was maintained at 60–70° for 38 hours. When cool, the resultant solid was recrystallized (acetone-hexane) to give 9.2 g. (59%) of product, M.P. 57–59°.

Analysis.—Calcd. for $C_{13}H_{19}NO_2$: C, 70.6; H, 8.7; N, 6.3. Found: C, 71.0; H, 8.8; N, 6.2.

Example 6.—N-(β-phenethyl)-β-hydroxypropionamide

To a cooled solution (5° aqueous) of 12.1 g. (0.1 mole) of β-phenethylamine, was added dropwise over 15 minutes 7.2 g. (0.1 mole) of freshly distilled β-propiolactone. After removal of water by vacuum distillation, the residue was distilled to give 10.7 g., B.P. 180° (.08 mm.), n_D^{20} 1.5483.

Analysis.—Calcd. for $C_{11}H_{15}NO_2$: N, 7.3. Found: N, 7.0.

Example 7.—Tablet formation

A granulation is prepared of

Lactose	parts 74
Starch	do 26

Water, a sufficient quantity.

The granulation is dried and screened.

	G.
Hydroxyamide	100
Lactose granulation	145
Magnesium stearate	5

are mixed well together and compressed into tablets weighing 250 mg. (diameter 9 mm.) and containing 100 mg. of hydroxyamide.

Other typical formulations include coated tablets, capsules, and sustained-release capsules containing 50–500 mg. of active ingredient per unit dose.

Example 8.—Elixers

An elixer is prepared containing per liter

Hydroxyamide	g	10.0
Ethyl alcohol	ml	150.0
Glycerin	ml	350.0
Sorbitol (70% solution)	ml	350.0
Benzoic acid	g	1.0
Saccharin sodium	g	0.2
Sucaryl sodium (cyclamate sodium and saccharin)	g	2.0
Coloring agent (FD & C Red No. 2)	g	0.02
Imitation raspberry flavor	ml	0.2
Spice vanilla	ml	0.02

Distilled water to 1000 ml.

The hydroxyamide is dissolved in about two-thirds of the ethanol and the glycerin and sorbitol are added. The benzoic acid and flavors are dissolved in the balance of the alcohol and combined with the first solution. The saccharin and Sucaryl are dissolved in a small amount of the water and coloring agent dissolved therein. The aqueous solution is then added to the alcohol solution, the balance of the water is added to bring the volume to 1 liter and after mixing and filtering an elixer is obtained containing 20 mg. of the hydroxyamide per ml. A unit dose of 15 ml. (1 tablespoon) thus contains 150 mg. of the hydroxyamide.

Among the additional formulations, which are readily preparable by familiar procedures, are suppositories weighing about 3 g. containing 150–300 mg. of hydroxyamide.

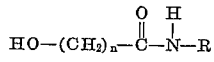
In the foregoing Examples 7 and 8, it will be understood that any of the hydroxyamides herein characterized as the essential active ingredients may be used, or that a mixture of these amides can be substituted as the active component, and that the amounts of active component can be suitably varied within the range of 50 to 500 mg., and preferably 100 to 400 mg. per dosage unit. In addition, other therapeutic agents may be added to these formulations if so desired.

Various changes and modifications in the procedures for preparing these hypocholesteremic hydroxyamides and incorporating the same into therapeutic compositions will

occur to those skilled in the art, and to the extent that such changes and modifications are embraced by the appended claims, it is to be understood that they constitute part of this invention.

Having described our invention, what we claim as new and desire to secure by Letters Patent is:

1. A method for lowering cholesterol levels which comprises administering to an animal a composition containing as an essential active ingredient a compound consisting of hydroxyamides having the following formula



wherein *n* is an integer from 1–4, and R is selected from the group consisting of C_4–C_5 alkyl and β-phenethyl, said administration being in amounts sufficient to substantially reduce the cholesterol level of the blood of the hypercholesteremic animal.

2. A method for lowering cholesterol levels as defined in claim 1 wherein the essential active ingredient is N-β-(β-phenethyl)glycolamide.

3. A method for lowering cholesterol levels as defined in claim 1 wherein the essential active ingredient is N-β-phenethyl-β-hydroxypropionamide.

4. A method for lowering cholesterol levels as defined in claim 1 wherein the essential active ingredient is N-β-phenethyl-δ-hydroxyvaleramide.

5. A method for lowering cholesterol levels as defined in claim 1 wherein the essential active ingredient is N-n-amyl-γ-hydroxybutyramide.

6. A method for lowering cholesterol levels as defined in claim 1 wherein the essential active ingredient is N-n-amyl-δ-hydroxyvaleramide.

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