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(54) Title: ALLYLMERCAPTOCAPTOPRIL COMPOUNDS AND USES THEREOF

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Compounds which are the reaction product of allicin and ACE-inhibiting compounds are useful in treating hypertension, elevated triglycerides, and elevated insulin.



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(54) Title: ALLYLMERCAPTOCAPTOPRIL COMPOUNDS AND USES THEREOF

(57) Abstract: Compounds which are the reaction product of allicin and ACE-inhibiting compounds are useful in treating hyper-  
tension, elevated triglycerides, and elevated insulin.



**WO 02/096871 A1**

## ALLYLMERCAPTOCAPTOPRIL COMPOUNDS AND USES THEREOF

## FIELD OF THE INVENTION

[0001] The present invention relates to allylmercaptocaptopril and derivatives thereof which are  
5 useful in treating hypertension, elevated triglycerides and hyperglycemia.

## BACKGROUND OF THE INVENTION

[0002] Captopril (D-3-mercapto-2-methylpropanoyl-L-proline) and related azetidine and proline derivatives are competitive  
10 inhibitors of angiotensin-converting enzyme (ACE) that block the conversion of angiotensin I to angiotensin II. Captopril and its derivatives are used as antihypertensive agents in treating most forms of hypertension (U.S. Patent No. 4,046,889, Ondetti et al, 1977; Thind, 1990; Cushman et al,  
15 1991; Migdalof et al, 1984; Materson et al, 1994).

[0003] Captopril contains a free sulfhydryl group and is quite stable in aqueous solution. However, in the blood or plasma of mammals, including humans, it is rapidly oxidized to its disulfide dimer and is involved in other disulfide  
20 exchange reactions. Captopril was found to bind covalently but reversibly to plasma proteins, cysteine, and glutathione (Migdalof et al, 1984).

[0004] Unfortunately, rather high doses of captopril are required to reduce blood pressure. This phenomenon may be  
25 because most of the captopril is used up in reactions with serum protein, to reduce -S-S bonds in proteins, and to form the mixed disulfide of protein-captopril. Thus, only a small amount of free captopril, or captopril recycled from the mixed disulfide with proteins, glutathione, and cysteine, remains  
30 for inhibiting ACE. Therefore, larger amounts of captopril are required as compared with other non-thiol containing ACE

inhibitors. This may explain the discrepancy between the *in vitro* and *in vivo* activity of captopril.

[0005] It has recently been shown that allicin (dithiosulfinate), a product of crushed garlic, which the present inventors prepared semi-synthetically (international patent WO 97/39115), also possesses antihypertensive properties. Other studies reported the remedial effects of allicin on cardiovascular risk factors, mainly on serum cholesterol, triglycerides levels, as well as lipoprotein balance, streak formation, and thrombogenesis in animals and in humans (Augusti et al, 1974; Eilat et al, 1995; Lawson, 1998; Abramovitz et al, 1999). Lipid lowering effect is one of the earliest established properties of garlic preparations, which also have a hypotensive effect as shown by Loeper et al (1921), which effect was confirmed by others in humans (Damrau, 1941; Petkov, 1979) and in animals (Chanderkar et al, 1973; Banerjee, 1979; Malik et al, 1981; Foushee et al, 1982; Ruffin et al, 1983; Auer et al, 1990; Agel et al, 1991).

[0006] Two possible mechanisms for the action of allicin have been suggested. One involves the antioxidant activity of allicin, while the other mechanism is that the particular structure of allicin, as activated disulfide, makes it a good candidate for interaction with the SH groups of proteins and low molecular weight thiols. Allicin, which is a very reactive compound, also disappears within a few minutes after being mixed with blood, due to its fast penetration through cell membranes, and its reaction with free thiol containing compounds, mainly reduced glutathione (GSH). From studies conducted by the present inventors, Rabinkov et al (1998) and Miron et al (2000), it appears that the active principal of allicin is the allylmercapto moiety (AM), which blocks thiol containing enzymes or reacts as a very efficient antioxidant.

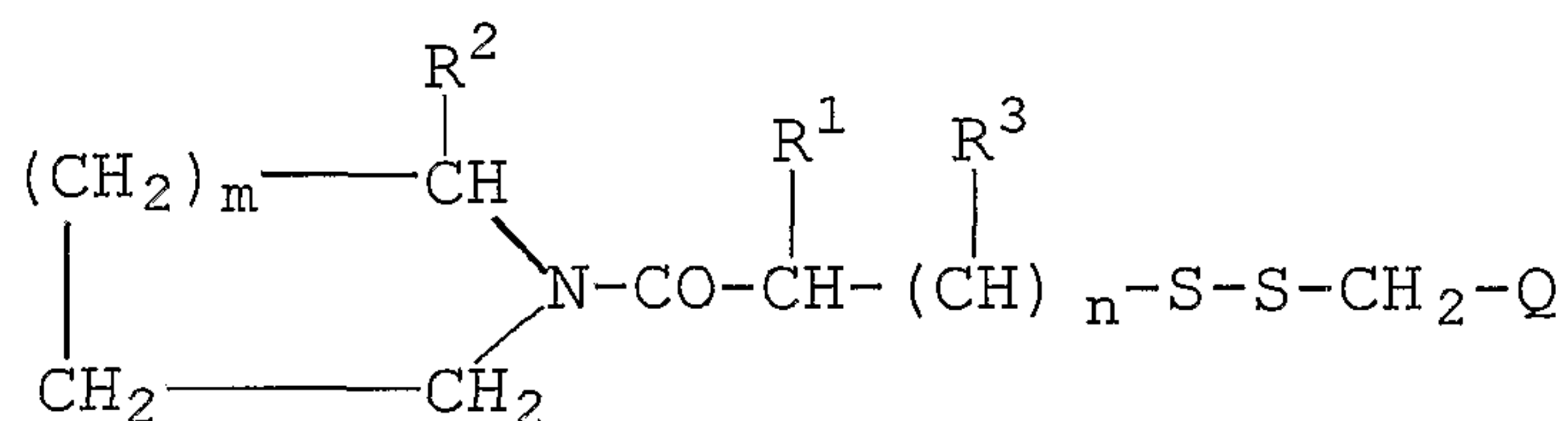
## SUMMARY OF THE INVENTION

[0007] It is an object of the present invention to overcome the aforesaid deficiencies in the prior art.

[0008] It is another object of the present invention to  
5 produce compounds which have hypotensive effects.

[0009] It is a further object of the present invention to produce compounds which lower triglycerides and insulin.

[0010] The compounds of the present invention have the formula



10

wherein:

$\text{R}^1$  and  $\text{R}^3$  are each hydrogen, lower alkyl, or phenyl-lower alkyl;

$\text{R}^2$  is hydrogen, hydroxy, lower alkyl or carboxyl;

15

$\text{Q}$  is  $-\text{CH}=\text{CH}_2$  or  $-\text{C}\equiv\text{CH}$ ;

$m$  is 0-10; and

$n$  is 0, 1, or 2.

[0011] Pharmaceutical compositions for treating hypertension include the compounds of the present invention,  
20 as active principle, along with a suitable pharmaceutically acceptable excipient or carrier. The active principle is present in an amount effective to reduce blood pressure.

[0012] The present invention further comprehends methods for treating hypertension by administering to a patient in  
25 need of reduction of hypertension an effective amount of a compound or composition of the present invention. Methods of treating elevated triglycerides and/or elevated insulin levels by administering such compounds or compositions are also encompassed by the present invention.

[0013] The present invention also includes a method for preparing the compounds of the present invention by reacting an HCE-inhibiting proline derivative compound with allicin or a derivative thereof.

5 [0014] The nature and objects of the present invention will be better understood upon consideration of the following detailed description in conjunction with the drawings.

#### BRIEF DESCRIPTION OF THE DRAWINGS

[0015] Figure 1 illustrates inhibition of papain activity  
10 by CPSSA at various concentrations.

[0016] Figure 2 shows inhibition of ACE by various concentrations of CPSSA.

[0017] Figure 3 illustrates the effect of CPSSA and captopril on blood pressure in rats.

15 [0018] Figure 4 shows the effect of CPSSA and captopril on triglycerides level in rats' sera.

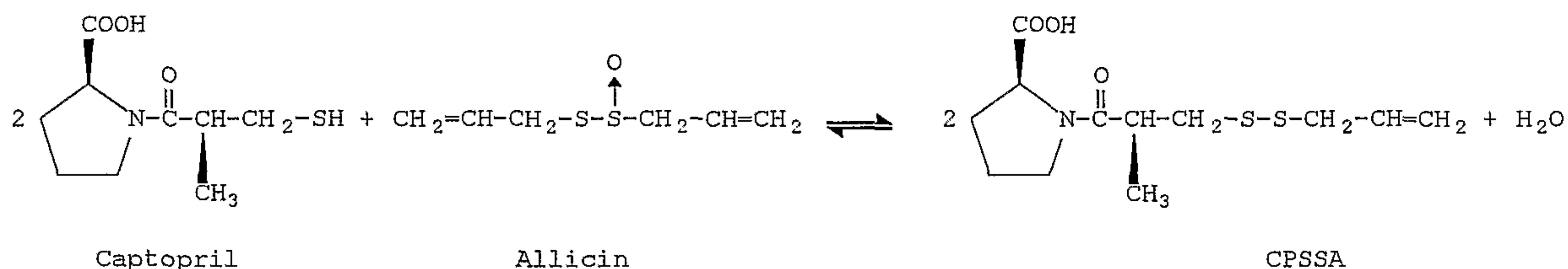
[0019] Figure 5 shows the effect of CPSSA and captopril on insulin level in rats' sera.

#### DETAILED DESCRIPTION OF THE INVENTION

20 [0020] The compounds of the present invention inhibit the conversion of the decapeptide angiotensin I to angiotensin II, and therefore they are useful in reducing or relieving angiotensin related hypertension, and in reducing triglycerides and insulin levels. The action of the enzyme  
25 renin on angiotensinogen, a pseudoglobulin in blood plasma, produces angiotensin I. Angiotensin I is converted by angiotensin converting enzyme (ACE) to angiotensin II, which is an active pressor substance which has been implicated as the causative agent in various forms of hypertension in many  
30 mammalian species. The compounds of the present invention intervene in the renin  $\longrightarrow$  angiotensin I  $\longrightarrow$  angiotensin II sequence by inhibiting angiotensin converting enzyme and

reducing or eliminating the formation of the pressor substance angiotensin II. Thus, by administering a composition containing at least one of the compounds of the present invention, angiotensin dependant hypertension can be alleviated.

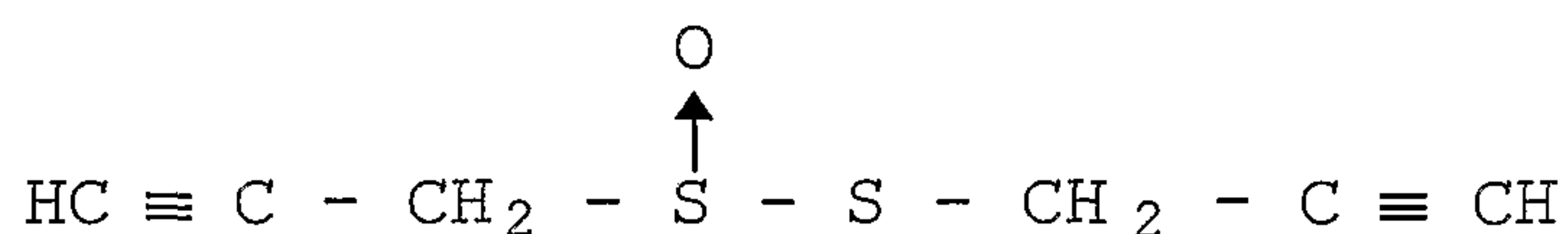
[0021] The compounds of the present invention combine the advantages of ACE-inhibiting azetidine and proline derivatives with the advantages of allicin, both of which are effective agents against hypertonia, each compound operating by a different mechanism. The product of the reaction between captopril *per se* and allicin is allylmercaptocaptopril (CPSSA), a non-symmetric disulfide (Scheme 1) which combines both the specific pharmacological activity of captopril and the beneficial properties of diallyldithiosulfinate (allicin).



Scheme 1

[0022] Additionally, dimers and derivatives of allicin, such as ajoine, which also react with thiols, can be used to prepare ACE-inhibiting compounds according to the present invention. For purposes of the present invention, "derivatives of allicin" refers to dimers, trimers, etc. as well as allicin compounds in which one or more of the carbon atoms are substituted which react with thiols.

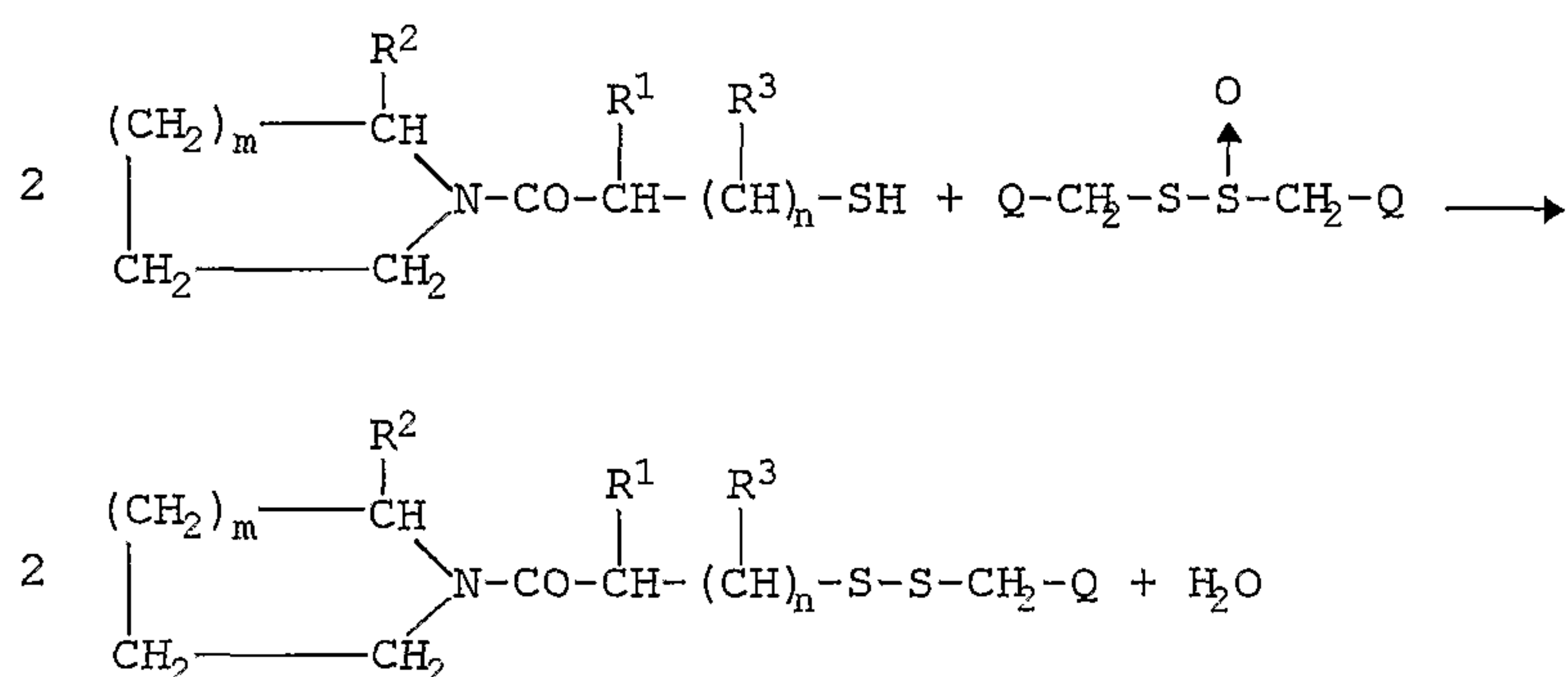
[0023] Compounds according to the present invention can also be prepared from the acetyleno (propargyl) analogue of allicin,



Dipropargyldithiosulfinate.

[0024] This propargyl analogue can be used to prepare the propargyl derivative of captopril, propargylmercaptocaptopril,  
5 as it performs the same thiolation reaction.

[0025] More generally, when Q is either allyl or propargyl, the following reaction occurs:



where

- 10  $\text{R}^1$  is hydrogen, lower alkyl or phenyl-lower alkyl;  
 $\text{R}^2$  is hydrogen, hydroxy, lower alkyl,  $\text{R}_3\text{CO}$ , or  
 carboxyl;  
 $\text{R}^3$  is hydrogen, lower alkyl or phenyl-lower alkyl;  
 $m$  is 0-10; and  
 15  $n$  is 0, 1, or 2.

[0026] The stereoisomers in which  $m$  is 1 and proline is in the L-form are especially preferred.

[0027] The lower alkyl groups represented by any of the variables include straight and branched chain  $\text{C}_1$ - $\text{C}_6$  hydrocarbon  
 20 radicals, such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, t-butyl, pentyl, isopentyl, hexyl, and the like. The lower alkoxy groups are of the same kind having from 1 to 7 carbon atoms linked to oxygen, such as methoxy, ethoxy, propoxy, isopropoxy, butoxy, isobutoxy, t-butoxy, and the  
 25 like. The  $\text{C}_1$ - $\text{C}_4$  members of this group, particularly the  $\text{C}_1$  and

C<sub>2</sub> members, of both types are preferred. Phenylmethyl is the preferred phenyl-lower alkyl group.

[0028] The lower alkanoyl groups are those which have acyl radicals of the lower (C<sub>2</sub>-C<sub>7</sub>) fatty acids, such as acetic, propionyl, butyryl, isobutyryl, and the like. Similarly, those lower alkanoyl groups having up to four carbon atoms, and particularly acetyl, are preferred.

[0029] Captopril and its derivatives can be prepared by methods disclosed in U.S. Patent No. 4,046,889, the entire contents of which being hereby incorporated by reference.

[0030] CPSSA and its derivatives and related compounds react very sluggishly with serum proteins where the thiol groups are mostly in the disulfide form. Thus, the compounds of the present invention are stable in blood or plasma of mammals and high doses of the compounds are not needed to produce an anti-hypertensive effect.

[0031] Compositions within the scope of the present invention include all compositions wherein the active ingredient is contained in an amount effective to achieve its intended purpose. While individual needs vary, determination of optimal ranges of effective amounts of each compound is within the skill of the art. Typical dosages comprise 0.01 to 100 mg/kg body weight. The preferred dosages comprising 0.1 to 100 mg/kg body weight. The most preferred dosages comprise 1 to 50 mg/kg body weight.

[0032] Pharmaceutical compositions for administering the active ingredients of the present invention preferably contain, in addition to the pharmacologically active compound, suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Preferably, the preparations, particularly

those preparations which are administered orally and which can be used for the preferred type of administration, such as tablets, dragees, and capsules, and also preparations which can be administered rectally, such as suppositories, as well  
5 as suitable solutions for administration by injection or orally, contain from about 0.01 to about 99 percent by weight, preferably from about 20 to 75 percent by weight, active compound(s), together with the excipient. For purposes of the present invention, all percentages are by weight unless  
10 otherwise indicated. In addition to the following described pharmaceutical composition, the compounds of the present invention can be formulated as inclusion complexes, such as cyclodextrin inclusion complexes.

[0033] Examples of pharmaceutically acceptable acid  
15 addition salts for use in pharmaceutical compositions according to the present invention include those derived from mineral acids, such as hydrochloric, hydrobromic, phosphoric, metaphosphoric, nitric, and sulfuric acids, and organic acids such as tartaric, acetic, citric, malic, lactic, fumaric,  
20 benzoic, glycolic, gluconic, succinic, and arylsulfonic, such as p-toluenesulfonic, acids.

[0034] The pharmaceutically acceptable carriers include vehicles, adjuvants, excipients, or diluents that are well known to those skilled in the art and which are readily  
25 available. It is preferred that the pharmaceutically acceptable carrier be one which is chemically inert to the active compounds and which has no detrimental side effects or toxicity under the conditions of use.

[0035] The choice of carrier is determined partly by the  
30 particular active ingredient, as well as by the particular method used to administer the composition. Accordingly, there is a wide variety of suitable formulations of the

pharmaceutical compositions of the present invention. Formulations can be prepared for oral, aerosol, parenteral, subcutaneous, intravenous, intraarterial, intramuscular, intraperitoneal, intratracheal, rectal, and vaginal administration.

[0036] Suitable excipients are, in particular, fillers such as saccharides, for example, lactose or sucrose, mannitol or sorbitol, cellulose preparations and/or calcium phosphates, for example, tricalcium phosphate or calcium hydrogen phosphate, as well as binders such as starch paste using, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, tragacanth, methyl cellulose, hydroxypropylmethylcellulose, sodium carboxymethylcellulose, and/or polyvinyl pyrrolidone.

[0037] Suitable formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form, such as water-soluble salts. In addition, suspensions of the active compounds as appropriate oily injection suspensions may be administered. Suitable lipophilic solvents or vehicles include fatty oils, for example, sesame oil, or synthetic fatty acid esters, for example, ethyl oleate or triglycerides. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, including, for example, sodium carboxymethyl cellulose, sorbitol, and/or dextran. Optionally, the suspension may also contain stabilizers.

[0038] Other pharmaceutically acceptable carriers for the active ingredients according to the present invention are liposomes, pharmaceutical compositions in which the active ingredient is contained either dispersed or variously present in corpuscles contained either dispersed or variously present in corpuscles consisting of aqueous concentric layers adherent

to lipid layers. The active ingredient may be present both in the aqueous layer and in the lipidic layer, inside or outside, or, in any event, in the non-homogeneous system generally known as a liposomic suspension.

5 [0039] The hydrophobic layer, or lipid layer, generally, but not exclusively, comprises phospholipids such as lecithin and sphingomyelin, steroids such as cholesterol, more or less ionic surface active substances such as dicetyl phosphate, stearylamine, or phosphatidic acid, and/or other materials of  
10 a hydrophobic nature.

[0040] The compounds may also be formulated for transdermal administration, for example in the form of transdermal patches so as to achieve systemic administration. Formulations suitable for oral administration can consists of liquid  
15 solutions such as effective amounts of the compound(s) dissolved in diluents such as water, saline, or orange juice; capsules, tables, sachets, lozenges, and troches, each containing a predetermined amount of the active ingredient as solids or granules; powders, suspensions in an appropriate  
20 liquid; and suitable emulsions. Liquid formulations may include diluents such as water and alcohols, e.g., ethanol, benzyl alcohol, and the polyethylene alcohols, either with or without the addition of a pharmaceutically acceptable surfactant, suspending agents, or emulsifying agents. Capsule  
25 forms can be of the ordinary hard- or soft-shelled gelatin type containing, for example, surfactants, lubricant, and inert fillers, such as lactose, sucrose, calcium phosphate, and corn starch. Tablet forms can include one or more of lactose, sucrose, mannitol, corn starch, potato starch,  
30 alginic acid, microcrystalline cellulose, acacia, gelatin, guar gum, colloidal silicon dioxide, croscarmellose sodium, talc, magnesium stearate, calcium stearate, zinc stearate,

stearic acid, and other preservatives, flavoring agents, and pharmaceutically acceptable disintegrating agents, moistening agents preservatives flavoring agents, and pharmacologically compatible carriers. Lozenge forms can comprise the active  
5 ingredient in a carrier, usually sucrose and acacia or tragacanth, as well as pastilles comprising the active ingredient in an inert base such as gelatin or glycerin, or sucrose and acacia. Emulsions and the like can contain, in addition to the active ingredient, such carriers as are known  
10 in the art.

[0041] Formulations suitable for parenteral administration include aqueous and non-aqueous, isotonic sterile injection solutions, which can contain anti-oxidants, buffers, bacteriostats, and solutes that render the formulation  
15 isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. The compounds can be administered in a physiologically acceptable diluent in a  
20 pharmaceutical carriers, such as a sterile liquid or mixture of liquids, including water, saline, aqueous dextrose and related sugar solutions, an alcohol such as ethanol, isopropanol, or hexadecyl alcohol, glycols such as propylene glycol or polyethylene glycol, glycerol ketals such as 2,2-  
25 dimethyl-1,3-dioxolane-4-methanol, ethers such as poly(ethylene glycol) 400, oils, fatty acids, fatty acid esters or glycerides, or acetylated fatty acid glycerides, without the addition of a pharmaceutically acceptable surfactants, such as soap or a detergent, suspending agent,  
30 such as carbomers, methylcellulose, hydroxypropylmethylcellulose, or carboxymethylcellulose, or emulsifying agents and other pharmaceutical adjuvants.

[0042] Oils which can be used in parenteral formulations include petroleum, animal, vegetable, or synthetic oils. Specific examples of oils include peanut, soybean, sesame, cottonseed, corn, olive, petrolatum, and mineral. Fatty acids  
5 can be used in parenteral formulations, including oleic acid, stearic acid, and isostearic acid. Ethyl oleate and isopropyl myristate are examples of suitable fatty acid esters. Suitable salts for use in parenteral formulations include fatty alkali metal, ammonium, and triethanolamine salts, and  
10 suitable detergents include cationic detergents such as dimethyl dialkyl ammonium halides, and alkyl pyridinium halides; anionic detergents such as dimethyl olefin sulfonates, alkyl, olefin, ether, and monoglyceride sulfates and sulfosuccinates; polyoxyethylenepolypropylene copolymers;  
15 amphoteric detergents such as alkyl-beta-aminopropionates and 2-alkyl-imidazoline quaternary ammonium salts; and mixtures thereof.

[0043] Parenteral formulations typically contain from about 0.5 to 25% by weight of the active ingredient in solution.  
20 Suitable preservatives and buffers can be used in these formulations. In order to minimize or eliminate irritation at the site of injection, these compositions may contain one or more nonionic surfactants having a hydrophilic-lipophilic balance (HLB) of from about 12 to about 17. The quantity of  
25 surfactant in such formulations ranges from about 5 to about 15% by weight. Suitable surfactants include polyethylene sorbitan fatty acid esters, such as sorbitan monooleate and the high molecular weight adducts of ethylene oxide with a hydrophobic base, formed by the condensation of propylene  
30 oxide with propylene glycol. The parenteral formulations can be present in unit dose or multiple dose sealed containers, such as ampules and vials, and can be stored in a freeze-dried

(lyophilized) condition requiring only the addition of the sterile liquid carrier, e.g., water, for injections immediately prior to use. Extemporaneous injection solutions and suspensions can be prepared from sterile powders, granules, and tablets of the kind previously described.

[0044] Additionally, the active ingredients can be formulated into suppositories by mixing the active ingredient with a variety of bases, including emulsifying bases or water-soluble bases. Formulations suitable for vaginal administration may be in the form of pessaries, tampons, creams, gels, pastes, foam, or spray formulations containing, in addition to the active ingredient, such carriers as are known in the art to be appropriate.

[0045] The active ingredients can be used as functionalized congeners for coupling to other molecules, such as amines and peptides. The use of such congeners provides for increased potency, prolonged duration of action, and prodrugs. Water solubility is also enhanced, which allows for reduction, if not complete elimination, of undesirable binding to plasma proteins and partition in to lipids. Accordingly, improved pharmacokinetics can be realized.

[0046] Any number of assays well known in the art, such as that of Carmel et al (1979), may be used to test whether a particular compound suspected of being an ACE inhibitor is effective as such.

[0047] In determining the dosages of the compounds to be administered, the dosage and frequency of administration is selected in relation to the pharmacological properties of the specific active ingredients. Normally, at least three dosage levels should be used. In toxicity studies in general, the highest dose should reach a toxic level but be sublethal for most animals in the group. If possible, the lowest dose

should induce a biologically demonstrable effect. These studies should be performed in parallel for each compound selected.

[0048] Additionally, the ID<sub>50</sub> level of the active ingredient  
5 in question can be one of the dosage levels selected, and the other two selected to reach a toxic level. The lowest dose that dose not exhibit a biologically demonstrable effect. The toxicology tests should be repeated using appropriate new doses calculated on the basis of the results obtained. Young,  
10 healthy mice or rats belonging to a well-defined strain are the first choice of species, an the first studies generally use the preferred route of administration. Control groups given a placebo or which are untreated are included in the tests. Tests for general toxicity, as outlined above, should  
15 normally be repeated in another non-rodent species, e.g., a rabbit or dog. Studies may also be repeated using alternate routes of administration.

[0049] Singe dose toxicity tests should be conducted in such a way that signs of acute toxicity are revealed and the  
20 mode of death determined. The dosage to be administered is calculated on the basis of the results obtained in the above-mentioned toxicity tests. It may be desired not to continue studying all of the initially selected compounds. Data on single dose toxicity, e.g., ID<sub>50</sub>, the dosage at which half of  
25 the experimental animals die, is to be expressed in units of weight or volume per kg of body weight and should generally be furnished for at least two species with different modes of administration. In addition to the ID<sub>50</sub> value in rodents, it is desirable to determine the highest tolerated dose and/or  
30 lowest lethal dose for other species, i.e., dog and rabbit.

[0050] When a suitable and presumably safe dosage level has been established as outlined above, studies on the drug's

chronic toxicity, its effect on reproduction, and potential mutagenicity may also be required in order to ensure that the calculated appropriate dosage range will be safe, also with regard to these hazards.

5 [0051] Pharmacological animal studies on pharmacokinetics revealing, e.g., absorption, distribution, biotransformation, and excretion of the active ingredient and metabolites are then performed. Using the results obtained, studies on human pharmacology are then designed. Studies of the  
10 pharmacodynamics and pharmacokinetics of the compounds in humans should be performed in healthy subjects using the routes of administration intended for clinical use, and can be repeated in patients. The dose-response relationship when different doses are given, or when several types of conjugates  
15 or combinations of conjugates and free compounds are given, should be studied in order to elucidate the dose-response relationship (dose vs. plasma concentration vs. effect), the therapeutic range, and the optimum dose interval. Also, studies on time-effect relationship, e.g., studies into the  
20 time-course of the effect and studies on different organs in order to elucidate the desired and undesired pharmacological effects of the drug, in particular on other vital organ systems, should be performed.

[0052] The compounds of the present invention are then  
25 ready for clinical trials to compare the efficacy of the compounds to existing therapy. A dose-response relationship to therapeutic effect and for side effects can be more finely established at this point.

[0053] The amount of compounds of the present invention to  
30 be administered to any given patient must be determined empirically, and will differ depending upon the condition of the patients. Relatively small amounts of the active

ingredient can be administered at first, with steadily increasing dosages if no adverse effects are noted. Of course, the maximum safe toxicity dosage as determined in routine animal toxicity tests should never be exceeded.

5 [0054] The compounds of the present invention can be administered in a single dose, or preferably two or four daily doses, provided on the basis of about 0.01 to 50 mg per kilogram per day, preferably about 0.1 to about 25 mg per kilogram per day, to reduce blood pressure. The compounds are  
10 preferably administered orally, but parenteral routes, such as subcutaneously, intramuscularly, intravenously, or intraperitoneally, can also be used.

[0055] The compounds of the present invention formulated in compositions such as tablets, capsules, or elixirs for oral  
15 administration, or in sterile solutions or suspensions for parenteral administration. About 1 to about 250 mg of a compound or mixture of compounds is compounded with a pharmaceutically acceptable vehicle, carrier, excipient, binder, preservative, stabilizer, flavor, etc., in a unit  
20 dosage form. The amount of active substance in these compositions or preparations is such that a suitable dosage in the range indicated is obtained.

#### MATERIALS AND METHODS

[0056] Pure allicin was produced by interaction of the  
25 synthetic substrate allicin with stabilized alliinase as described previously in Mirelman et al, international patent WO 97/39115. Fructose was purchased from Harlan, Teklad (Madison, WI). Captopril was purchased from Sigma, St. Louis, MO. Sprague-Dawley rats were purchased from ANILAB, Tal-  
30 Shahar, Israel.

[0057] Captopril, allylmercaptocaptopril, and allicin were separated by HPLC using an LKB HPLC system with an SP 4290

integrator (Spectraphysics). Separation was effected on a LiChrosorb RP-18 (7 mm) column using 60% methanol in water containing 0.1% formic acid or 0.05% trifluoroacetic acid as an eluant. The flow rate was 0.55 ml/minute.

5 [0058] CPSSA (0-5 mM) modification of activated and gel-filtered papain was conducted at room temperature in 50 mM sodium acetate, 2 mM EDTA pH 6.1. The inhibited papain was reactivated with 5 mM reduced glutathione.

[0059] The activity of angiotensin-converting enzyme was  
10 assayed according to Carmel et al (1979).

Animal experiments were conducted on Sprague-Dawley rats according to Reaven's model (Reaven et al, 1991), in which rats feeding on a fructose-enriched diet developed high blood pressure as well as high insulin and high triglyceride levels.

15 This model was previously used to test the effect of different angiotensin-converting enzyme inhibitors on metabolic parameters and blood pressure Erlich et al (1995), Erlich et al (1996). In the present study, the effects of CPSSA and captopril on blood pressure, as well as serum levels of  
20 triglycerides and insulin, were compared.

## RESULTS

### Example 1: Synthesis and Isolation of S-allylmercaptocaptopril (CPSSA)

[0060] The reaction of allicin and captopril was performed  
25 as follows:

[0061] Captopril (217 mg, 1 mmole) in 7.5 ml water pre-adjusted to pH 5.5, was added dropwise to allicin (90 mg, 0.55 mmole) dissolved in 3 ml absolute ethanol. The reaction mixture was magnetically stirred for 15-20 minutes at room  
30 temperature. Excess allicin was extracted by ether, and the water phase was acidified with HCL and extracted with ethyl acetate. The organic phase was dried by rotor evaporation,

re-dissolved either in ethanol and dried by Speed Vac centrifugation or re-dissolved in water and dried by lyophilization. The yield of the reaction was 90%. The reaction product was detected by HPLC analysis (RT 14.1 minutes). The structure was confirmed by NMR and MS.

**Example 2: SH Modification of Papain**

[0062] Incubation of papain with allylmercaptocaptopril caused a significant decrease of enzyme activity. The degree of inhibition was concentration dependent. The activity of the inactivated papain could be restored by reduced glutathione.

[0063] Activated papain was mixed with various concentrations of CPSSA for 10 minutes at room temperature in 50 mM sodium acetate, 2 mM EDTA buffer, pH 6.5. The residual enzyme activity was measured by monitoring the rate of hydrolysis of the substrate benzoyl arginine-p-nitro anilide at 382 nm. The results are shown in Figure 1.

**Example 3: Effect of CPSSA on Angiotensin Converting Enzyme Activity in Vitro**

[0064] In order to determine if CPSSA reacts in a similar way to captopril, which inhibits the activity of angiotensin converting enzyme, serial dilutions of CPSSA were prepared. Samples were added to a reference serum and the activity of the enzyme was monitored as shown in Figure 2. The inhibition was concentration dependant and essentially does not differ from the inhibition profile obtained with captopril.

**Example 4: Effect of CPSSA on Blood Pressure, Serum Triglycerides, Insulin Concentrations, and Body Weight**

[0065] Model Sprague-Dawley male rates, five rats/group, 220-240 g body weight, were fed with a fructose-rich diet for three weeks, followed by two weeks of treatment with either

CPSSA or captopril predissolved in the drinking water in doses of CPSSA of 40 and 57 mg/kg and 80 mg/kg captopril, together with the fructose-rich diet. The control group was fed only the fructose-rich diet. Measurements of body weight, blood pressure, serum levels of triglycerides and insulin were conducted at 0, 3, and 5 weeks into the treatment. As can readily be seen from Figures 3-5, CPSSA significantly decreased blood pressure (Figure 3) and reduced the serum levels of triglycerides (Figure 4) and insulin (Figure 5) to near normal levels once administration of CPSSA occurred. Similar results were obtained with captopril with doses that were nearly double that of CPSSA. CPSSA, like captopril, decreased serum insulin levels to normal values in model animals (Figure 5), but CPSSA appears to be more active than captopril in decreasing levels of triglycerides (Figure 4). No effects on body weight were found in either treatment.

[0066] Pharmaceutical compositions according to the present invention can be administered by any convenient route, including parenteral, subcutaneous, intravenous, intramuscular, intraperitoneal, or transdermal. Alternatively or concomitantly, administration may be by the oral route. The dosage administered depends upon the age, health, and weight of the recipient, nature of concurrent treatment, if any, and the nature of the effect desired.

[0067] The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying current knowledge, readily modify and/or adapt for various applications such specific embodiments without undue experimentation and without departing from the generic concept. Therefore, such adaptations and modifications should and are intended to be comprehended within the meaning and range of equivalents of

the disclosed embodiments. It is to be understood that the phraseology or terminology employed herein is for the purpose of description and not of limitation. The means and materials for carrying out various disclosed functions may take a  
5 variety of alternative forms without departing from the invention. Thus, the expressions "means to..." and "means for..." as may be found in the specification above and/or in the claims below, followed by a functional statement, are intended to define and cover whatever structural, physical,  
10 chemical, or electrical element or structures which may now or in the future exist for carrying out the recited function, whether or not precisely equivalent to the embodiment or embodiments disclosed in the specification above; and it is intended that such expressions be given their broadest  
15 interpretation.

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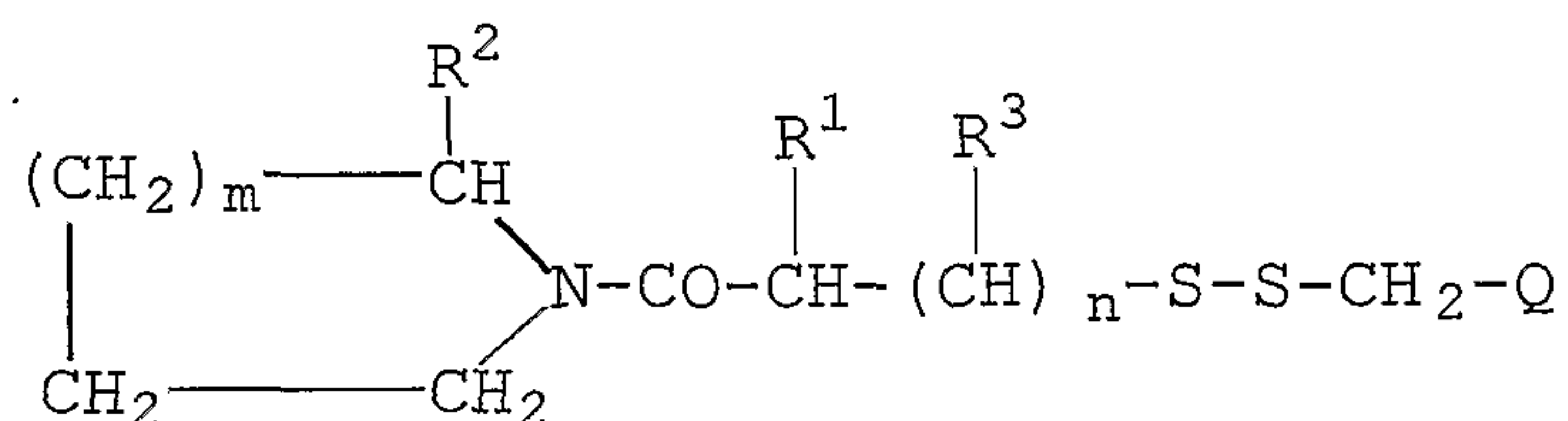
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WHAT IS CLAIMED IS:

1. Compounds of the formula:



where

- 5             $\text{R}^1$  is hydrogen, lower alkyl or phenyl-lower alkyl;  
               $\text{R}^2$  is hydrogen, hydroxy, lower alkyl,  $\text{R}_3\text{CO}$ , or  
              carboxyl;  
               $\text{R}^3$  is hydrogen, lower alkyl or phenyl-lower alkyl;  
               $m$  is 0-10;  
 10            $n$  is 0, 1, or 2; and  
               $\text{Q}$  is  $-\text{CH}=\text{CH}_2$  or  $-\text{C}\equiv\text{CH}$ .
2. The compound according to claim 1, wherein  $m$  is 1  
 to 5.
3. The compound according to claim 1, wherein  $\text{R}^1$  is  
 15 hydrogen or lower alkyl;  $\text{R}^2$  and  $\text{R}^3$  are each hydrogen.
4. The compound according to claim 1, wherein  $\text{R}^2$  and  
 $\text{R}^3$  are each hydrogen.
5. The compound according to claim 1, wherein  $\text{R}^2$  is  
 hydrogen.
- 20           6. The L-form of the compound of claim 1.
7. The compound according to claim 1, wherein  $n$  is  
 1.
8. The compound according to claim 1, wherein  $\text{R}^1$  is  
 hydrogen or lower acetyl.
- 25           9. The compound according to claim 1, wherein  $\text{R}^1$  is  
 hydrogen or methyl.
10. The compound according to claim 1, wherein  $n$  is  
 0.
11. The compound according to claim 7, wherein  $\text{R}^1$ ,  
 30  $\text{R}^2$ , and  $\text{R}^3$  are each hydrogen.

12. The compound according to claim 7, wherein  $R^1$  is methyl and  $R^2$  and  $R^3$  are each hydrogen.

13. The compound according to claim 11, wherein m is 1 and the proline is in the L-form.

5 14. The compound according to claim 12, wherein m is 1 and the proline is in the L-form.

15. The compound according to claim 13, wherein m is 1 and the proline is in the L-form.

10 16. The compound according to claim 1, wherein Q is  $-C=CH_2$ .

17. The compound according to claim 1, wherein Q is  $-C\equiv CH$ .

15 18. A method for treating hypertension comprising administering to a patient in need thereof an effective amount of a compound according to claim 1.

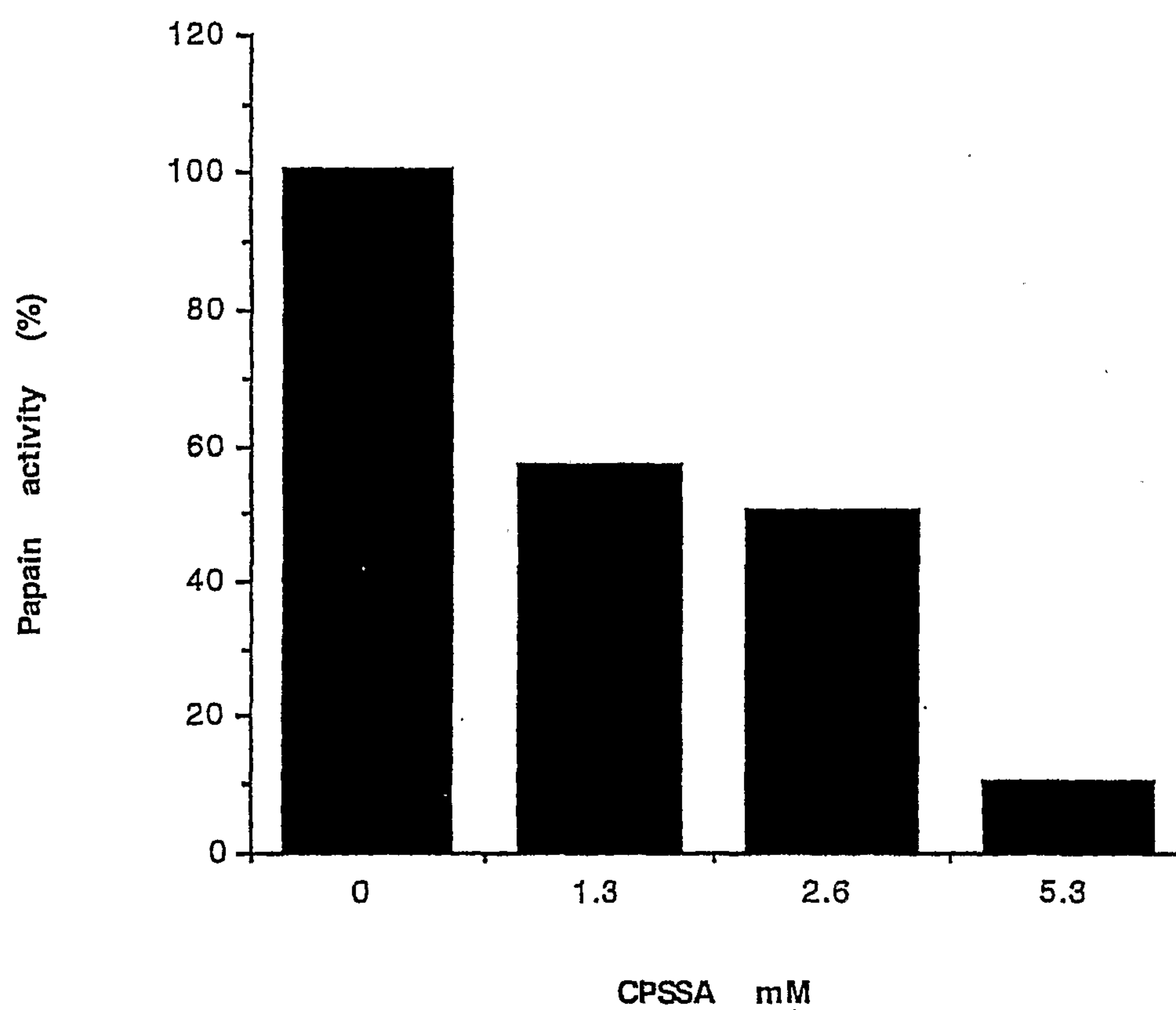
19. A pharmaceutical composition for treating hypertension comprising an effective amount to reduce blood pressure of at least one compound according to claim 1 and a pharmaceutically acceptable carrier.

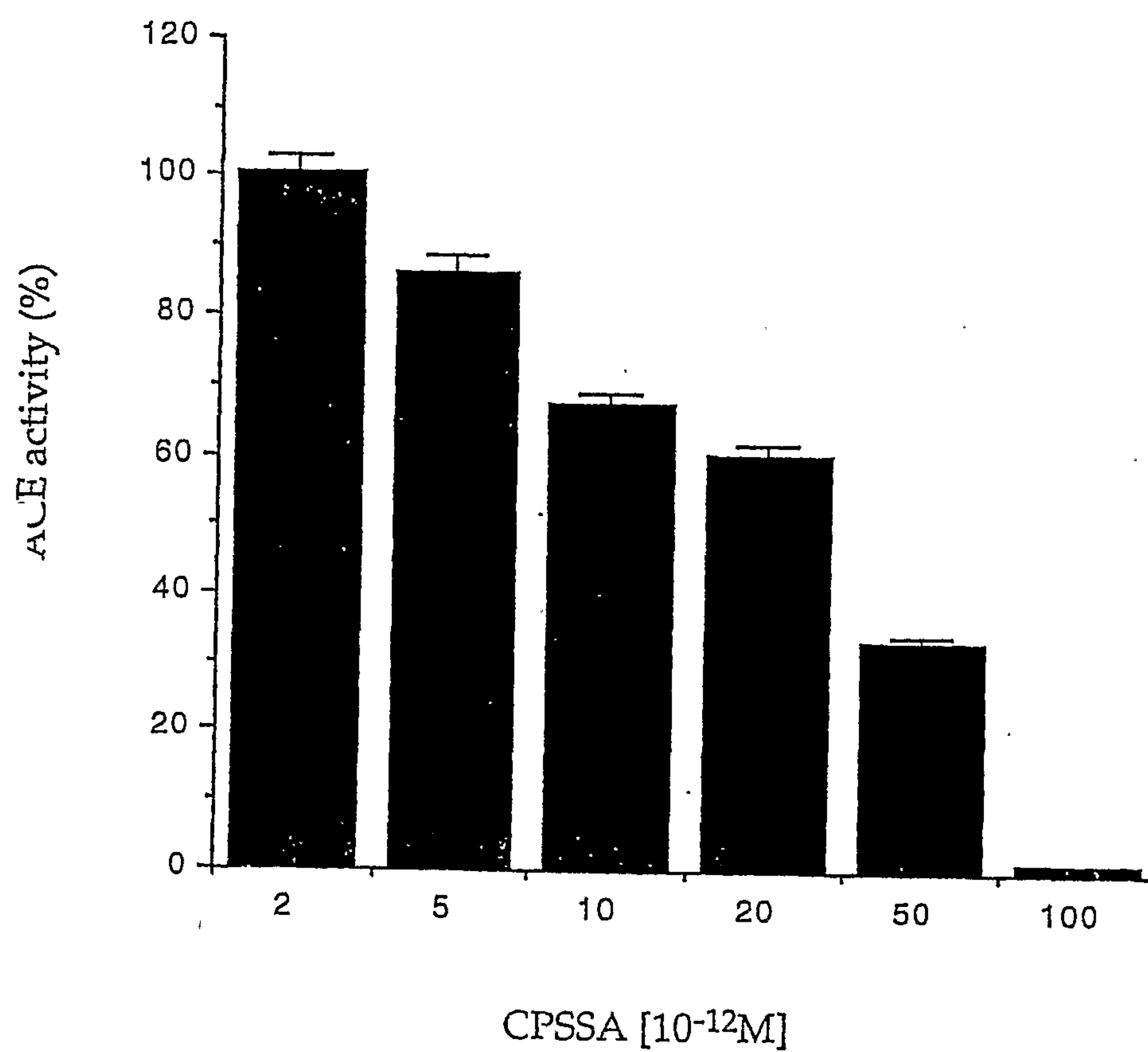
20 20. A method for preparing compounds for treating hypertension comprising reacting an ACE-inhibiting proline derivative compound with allicin or a derivative thereof.

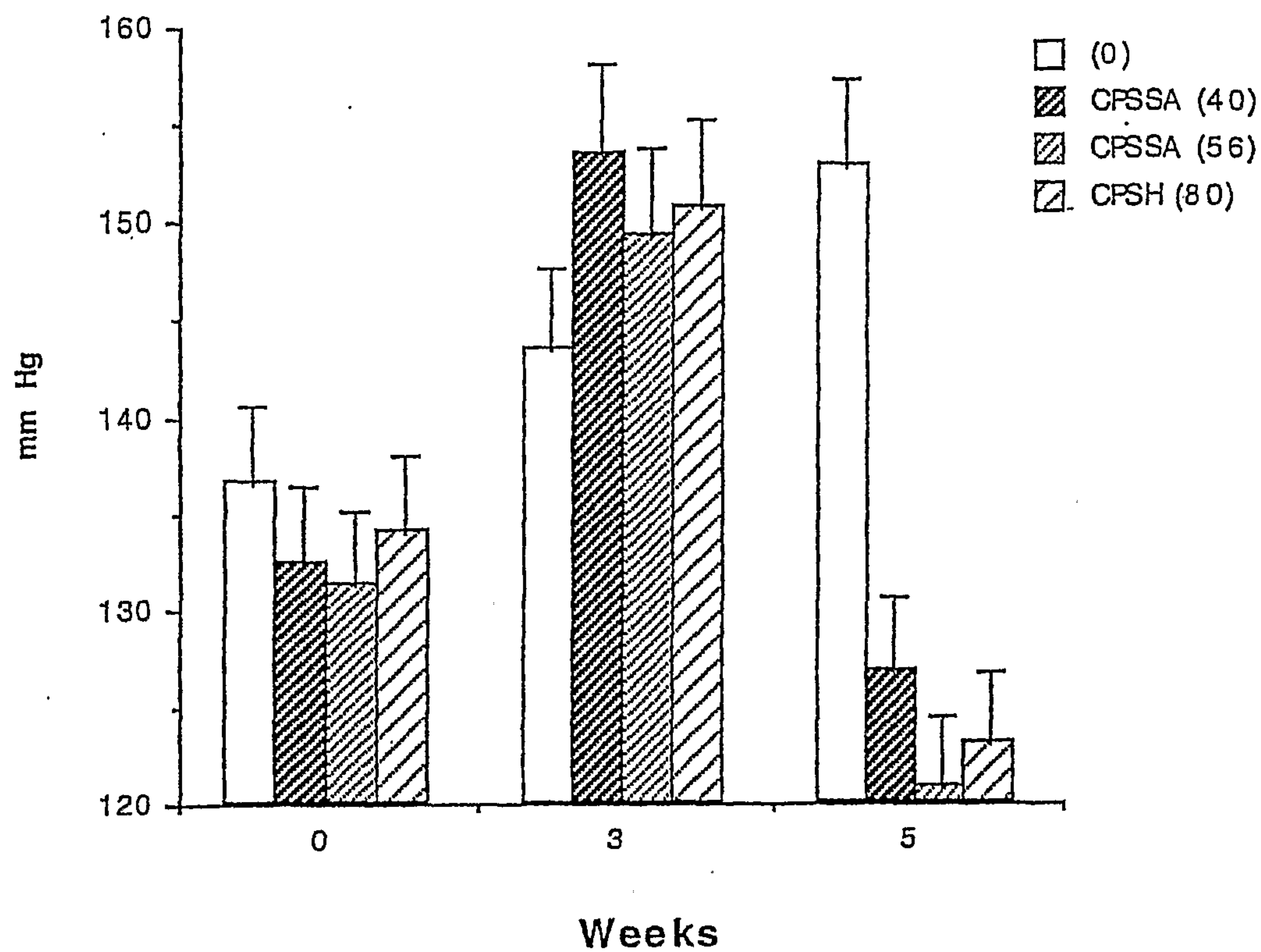
21. The method according to claim 20, wherein the ACE-inhibiting proline derivative is captopril.

25 22. A method for treating elevated triglycerides comprising administering to a patient in need thereof an effective amount of a compound according to claim 1.

30 23. A method for treating elevated insulin levels comprising administering to a patient in need thereof an effective amount of a compound according to claim 1.

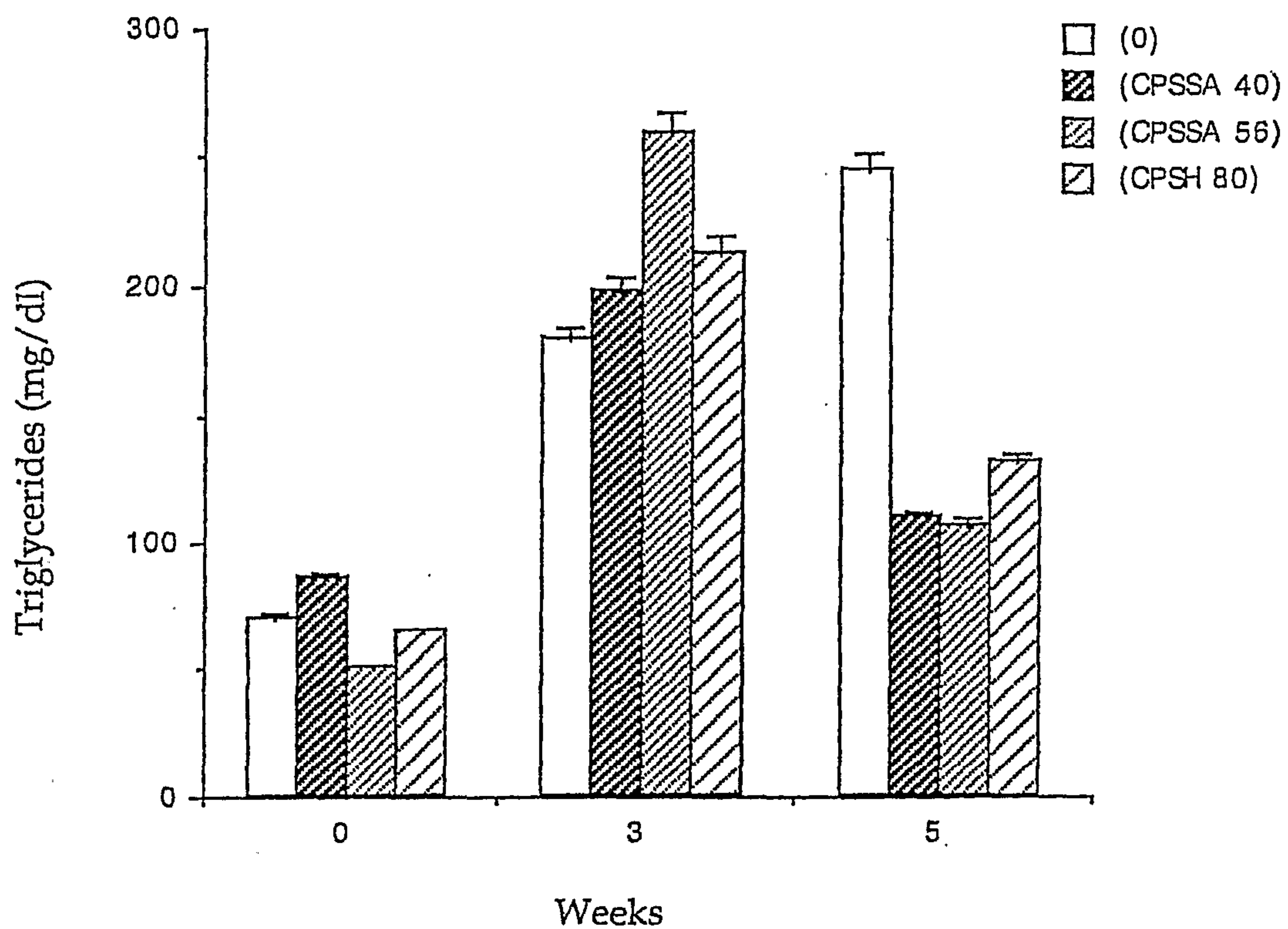
**FIGURE 1**

**FIGURE 2**



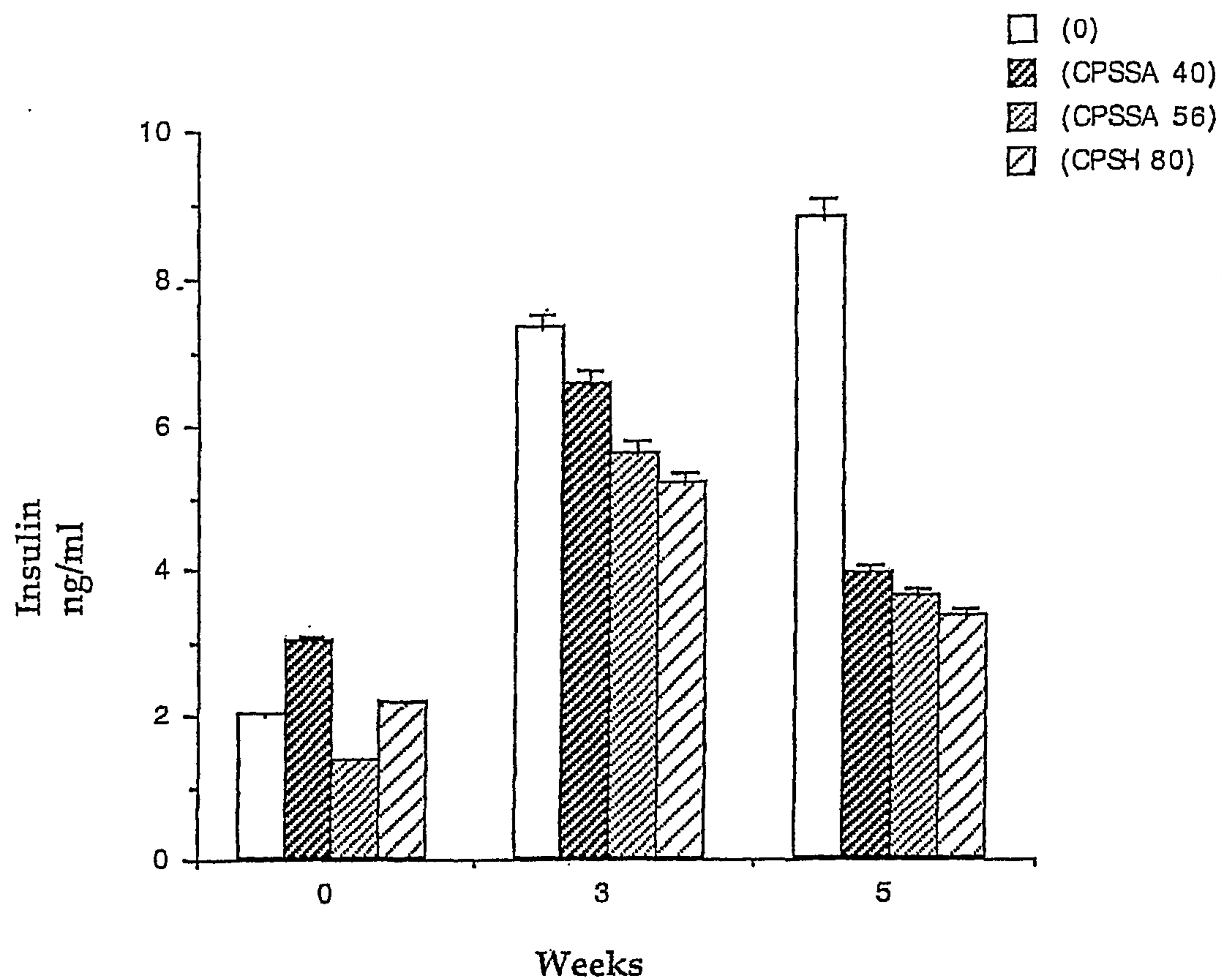
The effect of CPSSA and captopril on blood pressure in rats. Rats were fed 5 weeks with fructose rich diet. The rats, after 3 weeks of diet, were treated with either CPSSA 40 mg/kg [  ], 56mg/kg [  ] or captopril 80 mg/kg [  ] in the drinking water. The control group [  ] was fed with fructose rich diet only.

**FIGURE 3**



The effect of CPSSA and captopril on triglycerides level in rats sera. Rats were fed 5 weeks with fructose rich diet. The rats, after 3 weeks of diet, were treated with either CPSSA 40 mg/kg [▨], 56mg/kg [▩] or captopril 80 mg/kg [▧] in the drinking water. The control group [□] was fed with fructose rich diet only.

**FIGURE 4**



The effect of CPSSA and captopril on insulin level in rats sera. Rats were fed 5 weeks with fructose rich diet. The rats, after 3 weeks of diet, were treated with either CPSSA 40 mg/kg [▨], 56mg/kg [▩] or captopril 80 mg/kg [▧] in the drinking water. The control group [□] was fed with fructose rich diet only.

**FIGURE 5**