CONVENTION

AUSTRALIA

Patents Act

618595

APPLICATION FOR A STANDARD PATENT

Suntory Limited
1-40, Dojimahama, 2-chome, Kita-ku, Osaka-shi, Osaka, JAPAN

hereby applies for the grant of a standard patent for an invention entitled:

DERIVATIVE OF CAFFEIC ACID AND PHARMACEUTICAL COMPOSITION CONTAINING THE SAME

which is described in the accompanying complete specification.

Details of basic application(s):-

106274	JAPAN	28 April 1988
55867	JAPAN	8 March 1989

Address for Service:

PHILLIPS ORMONDE & FITZPATRICK
Patent and Trade Mark Attorneys
367 Collins Street
Melbourne 3000 AUSTRALIA

DATED this TWENTY EIGHTH day of APRIL 1989

PHILLIPS ORMONDE & FITZPATRICK
Attorneys for:
Suntory Limited

By:

Our Ref : 131419
POF Code: 1594/33344

M008580 28/04/89

TF/S-34-182 AU
[illegible]-182 AU-1

AUSTRALIA

Patents Act

DECLARATION FOR A PATENT APPLICATION

▼ INSTRUCTIONS
(a) Insert "Convention" if applicable
(b) Insert FULL name(s) of applicant(s)

In support of the (a) Convention application made by
(b)
SUNTORY LIMITED

(c) Insert "of addition" if applicable
(d) Insert TITLE of invention

(hereinafter called "applicant(~~s~~)" for a patent (c) for an invention entitled (d)

DERIVATIVE OF CAFFEIC ACID AND PHARMACEUTICAL COMPOSITION CONTAINING THE SAME

(e) Insert FULL name(s) AND address(es) of declarant(s) (See headnote *)

I/~~We~~ (e)
Keizo SAJI, President of SUNTORY LIMITED of 1-40, Dojimahama 2-chome, Kita-ku, Osaka-shi, Osaka, Japan

do solemnly and sincerely declare as follows:

1. ~~I am/We are the applicant(s).~~
(or, in the case of an application by a body corporate)
1. I am/~~We are~~ authorized to make this declaration on behalf of the applicant(~~s~~).
2. ~~I am/We are the actual inventor(s) of the invention.~~
(or, where the applicant(s) is/are not the actual inventor(s))
2. (f)

(f) Insert FULL name(s) AND address(es) of actual inventor(s)

P. T. O.

(g) Recite how applicant(s) derive(s) title from actual inventor(s) (See headnote **)

~~is~~/are the actual inventor(s) of the invention and the facts upon which the applicant(~~s~~) is/~~are~~ entitled to make the application are as follows:
(g)
Applicant is the assignee of the invention from the actual inventors.

(Note: Paragraphs 3 and 4 apply **only** *to Convention applications)*

(h) Insert country, filing date, and basic applicant(s) for the/or EACH basic application

3. The basic application(s) for patent or similar protection on which the application is based ~~is~~/are identified by **country, filing date, and basic applicant(s)** as follows:
(h)
Japan on April 28, 1988 and March 8, 1989
by SUNTORY LIMITED

4. The basic application(s) referred to in paragraph 3 hereof ~~was~~/were the first application(s) made in a Convention country in respect of the invention the subject of the application.

(k) Insert PLACE of signing
(l) Insert DATE of signing
(m) Signature(s) of declarant(s)
Note: No legalization or other witness required

Declared at (k) Osaka, Japan
Dated (l) April 20, 1989
(m) SUNTORY LIMITED
Keizo Saji
President

To: The Commissioner of Patents

P18/7/78

PHILLIPS ORMONDE & FITZPATRICK
Patent and Trade Mark Attorneys
367 Collins Street
Melbourne, Australia

2. Inventors:

Hidetsura CHO	:	15-14, Terada-cho, Ibaraki-shi, Osaka, Japan
Masaru UEDA	:	1-15-2, Motomachi, Urawa-shi, Saitama-ken, Japan
Mie TAMAOKA	:	15, Furuichiba, Utano-cho, Uda-gun, Nara-ken, Japan
Mikiko HAMAGUCHI	:	1-62-12, Daiwa-nishi, Kawanishi-shi, Hyogo-ken, Japan
Seiitsu MUROTA	:	3-8-11-301, Koishikawa, Bunkyo-ku, Tokyo, Japan
Ikuo MORITA	:	3-12-27-320, Hasune, Itabashi-ku, Toyo, Japan

(12) PATENT ABRIDGMENT (11) Document No. AU-B-33813/89
(19) AUSTRALIAN PATENT OFFICE (10) Acceptance No. 618595

(54) Title
DERIVATIVE OF CAFFEIC ACID AND PHARMACEUTICAL COMPOSITION CONTAINING THE SAME

International Patent Classification(s)
(51)[4] C07C 121/75 A61K 031/38 A61K 031/44 C07D 213/30
C07D 233/64 C07D 333/16 A61K 031/275 A61K 031/415
(51)[5] C07C 255/41

(21) Application No. : 33813/89 (22) Application Date : 28.04.89

(30) Priority Data

(31) Number	(32) Date	(33) Country
63-106274	28.04.88	JP JAPAN
1-55867	08.03.89	JP JAPAN

(43) Publication Date : 02.11.89

(44) Publication Date of Accepted Application : 02.01.92

(71) Applicant(s)
SUNTORY LIMITED

(72) Inventor(s)
HIDETSURA CHO; MASARU UEDA; MIE TAMAOKA; MIKIKO HAMAGUCHI; SEIITSU MUROTA; IKUO MORITA

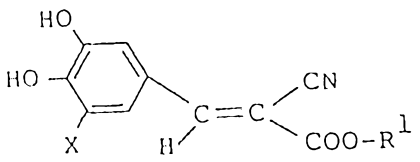
(74) Attorney or Agent
PHILLIPS ORMONDE & FITZPATRICK , 367 Collins Street, MELBOURNE VIC 3000

(56) Prior Art Documents
AU 27360/88 C07C 125/75
AU 81879/87 C07C 125/75

(57) Since the caffeic acid derivatives of the present invention possess inhibitory activity against 12-lipoxygenase as well as 5-lipoxygenase, they are useful as remedies for circulatory diseases, for example, the prevention of arteriosclerosis.

CLAIM

1. A caffeic acid derivative of the general formula (I) and pharmaceutically permissible salts thereof:



wherein X is hydrogen atom or hydroxy; R^1 is hydrogen atom, a straight or branched alkyl or alkenyl having 1 to 20 carbon atoms, or a group of the formula:

$$-(CH_2)_n-Z-R^2$$

wherein n is an integer of 1 to 10; Z is oxygen atom, vinylene residue or a single bond, and R^2 is a phenyl which may be substituted with up to 3 substituents selected from hydroxy, a

.../2

lower alkoxy or a halogen atom or R^2 is a heterocyclic residue; provided that X and R^1 each cannot be a hydrogen atom simultaneously.

4. A pharmaceutical composition used as a remedy for circulatory diseases which comprises a pharmaceutically effective amount of a compound of Claim 1.

AUSTRALIA

Patents Act

618595

COMPLETE SPECIFICATION
(ORIGINAL)

Class Int. Class

Application Number:
Lodged:

Complete Specification Lodged:
Accepted:
Published:

Priority

Related Art:

Applicant(s):

Suntory Limited
1-40, Dojimahama 2-chome,
Kita-ku, Osaka-shi, Osaka, Japan

Address for Service is:

PHILLIPS ORMONDE & FITZPATRICK
Patent and Trade Mark Attorneys
367 Collins Street
Melbourne 3000 AUSTRALIA

Complete Specification for the invention entitled:

DERIVATIVE OF CAFFEIC ACID AND PHARMACEUTICAL COMPOSITION CONTAINING THE SAME

Our Ref : 131419
POF Code: 1594/33344

The following statement is a full description of this invention, including the best method of performing it known to applicant(s):

6006

DERIVATIVE OF CAFFEIC ACID AND PHARMACEUTICAL COMPOSITION CONTAINING THE SAME

The present invention relates to a novel derivative of caffeic acid which possesses 12-lipoxygenase inhibitory activity and is useful for the prevention and remedy of circulatory diseases such as arteriosclerosis, and to a pharmaceutical composition containing said derivative.

Leukotriene, which is considered to be a cause of allergic diseases such as asthma, is produced from arachidonic acid *via* 5-hydroperoxyeicosatetraenoic acid (5-HPETE) which is generated by the action of 5-lipoxygenase and 5-hydroxyeicosatetraenoic acid (5-HETE) as intermediates. On the basis of this fact, a number of compounds exhibiting inhibitory activity against 5-lipoxygenase have been reported in connection with the development of agents for treating circulatory diseases.

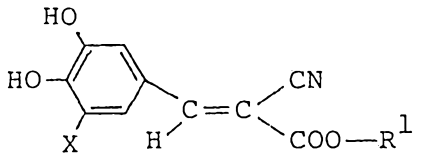
On the other hand, it is also known that 12-hydroperoxyeicosatetraenoic acid (12-HPETE) and 12-hydroxyeicosatetraenoic acid (12-HETE) are produced from arachidonic acid by another converting enzyme, 12-lipoxygenase. With respect to the biological activity of these compounds, Tada et al. reported that 12-HETE is related to the outbreak of ischemic heart disease (Cardiovascular Research, vol. 21, No. 8, 551 - 558, 1987), and Murota et al. reported that 12-HETE exhibited harmful activity against endothelial cells and enhanced the migration of smooth muscle cells in the vascular medial membrane to result in ingravescence of circulatory diseases such as arteriosclerosis and nephritis (Chiryogaku, vol. 13, No. 6, 785 - 788, 1984).

These facts suggest that compounds capable of inhibiting 12-lipoxygenase may be expected to be useful in the treatment of circulatory diseases; among such compounds baicalein isolated from *Scutellaria baicalensis* have been reported as a naturally occurring compound while the derivatives of tropolone (Japanese Patent Laid-Open

No. 228414/1985) and those of naphthalene (Japanese Patent Laid-Open Nos. 251640/1986, 251641/1986 and 251642/1986) have been reported as synthetic compounds. Since their biological activity is not sufficient, however, the development of novel inhibitors is required.

Taking this situation into consideration, the present inventors completed the present invention by screening a wide range of compounds possessing 12-lipoxygenase inhibitory activity and found that derivatives of caffeic acid of the general formula (I) are as active as or more active than the aforementioned inhibitors against 12-lipoxygenase, and also that these derivatives have low toxicity and that synthesis thereof is easy.

According to the present invention, derivatives of caffeic acid of the general formula (I) are provided:



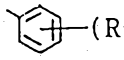
(I)

wherein X is hydrogen atom or hydroxy; R^1 is hydrogen atom, a straight or branched alkyl or alkenyl having 1 to 20 carbon atoms, or a group of the formula:

$-(CH_2)_n-Z-R^2$

with the proviso that R^1 and X are not both hydrogen

wherein n is an integer of 1 to 10; Z is oxygen atom, vinylene residue or a single bond; and R^2 is a substituted or unsubstituted phenyl or heterocyclic residue; as well as their pharmaceutically acceptable salts, and pharmaceutical compositions containing said derivative. Examples of R^1 and the general formula (I) include, for instance, methyl, ethyl, propyl, isopropyl, n-butyl, n-pentyl, n-hexyl, eicosyl and the like for alkyls; allyl, 2-butenyl, 3-butenyl, 4-pentenyl, 4-hexenyl, 5Z,8Z,11Z,14Z-eicosatetraenyl and the like as alkenyls.

Examples of R^2 in the group $-(CH_2)_n-Z-R^2$ include, for instance,  ,  ,  or  and the like;

wherein R^3 is hydroxy, lower alkoxy or halogen atom; m is 0 or an integer of 1 to 3.

The compounds of the present invention may be synthesized by the methods explained below.

As the first step an alcohol that is commercially available or has been prepared by means of a conventional method and that has the general formula (II):

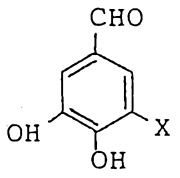
$$R^1\text{-}OH \qquad (II)$$

wherein R^1 has the meaning given above, is protected as necessary if R^1 contains functional groups and is then condensed with cyanoacetic acid by a condensing reagent such as 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide in the presence of a base such as 4-dimethylaminopyridine, pyridine or piperidine in an inert solvent such as N,N-dimethylformamide to obtain an ester of cyanoacetic acid of the general formula (III):

$$\begin{array}{l} CH_2\text{-}COOR^{1\prime} \\ | \\ CN \end{array} \qquad (III)$$

wherein $R^{1\prime}$ is R^1 as defined above or R^1 with protected substituents.

The cyanoacetic acid ester (III) obtained from the foregoing step is coupled with a derivative of benzaldehyde of the general formula (IV):



(IV)

wherein X has the meaning given above, in an inert solvent such as benzene or toluene in the presence of a base catalyst such as pyridine or piperidine under the traditional conditions for Knoevenagel condensation and is deprotected by an appropriate method if the substituents in R^1 have been protected, whereby a desired derivative of caffeic acid of the general formula (I), is obtained.

Purification of the compounds of the present invention obtained in the above manner is performed by filtering off the precipitated crystalline after cooling the reaction

mixture, or by silica gel column chromatography.

Suitable examples of the starting material of the general formula (II) include methanol, ethanol, isopropanol, (5Z,8Z,11Z,14Z)-eicosatetraenol, benzyl alkylene alcohols with or without substituents on the benzene ring, such as phenethyl alcohol, heterocyclic alkylene alcohols, such as 2-(2-thienyl)ethanol, phenyl vinyl alcohols such as 3-phenyl-2-propenol or phenoxy alkylene alcohols such as 2-phenoxyethanol.

The caffeic acid derivatives synthesized may be converted into pharmaceutically acceptable salts thereof as required. For example, sodium, potassium or calcium salts may be used as a medicine.

Since the caffeic acid derivatives of the present invention possess inhibitory activity against 12-lipoxygenase as well as 5-lipoxygenase, they are useful as remedies for circulatory diseases, for example, the prevention of arteriosclerosis.

When applied as remedies for circulatory diseases, the caffeic acid derivatives of the present invention or pharmaceutically permissible salts thereof may be administered orally or parenterally in an appropriate dosage form such as capsule, tablet, or injection, alone or together with known innocuous excipients. For example, these dosage forms may be provided by adopting the following procedures. In order to provide capsules, the original compound is pulverized, mixed with an excipient such as lactose, starch, a derivative thereof or a cellulose derivative, and packed in gelatin capsules. In the case of tablets, a binder such as sodium carboxymethylcellulose, alginic acid or gum arabic and water are added to the compound in addition to the excipient mentioned above, and mixed together by kneading, formed into granules as required, and then a lubricant such as talc or stearic acid is also added. The mixture is formed into tablets using a tablet machine. Injections for parenteral administration are prepared by dissolving the compound of the present invention in sterilized distilled water or sterilized saline together with a solubilizer and

packing the solution in sealed ampules. Stabilizers or buffers may be included as required.

Although the effective dose of remedies for circulatory diseases will vary depending on the type and seriousness of the disease, the administration method and the physical condition of the patient, a sufficient amount to suppress the symptoms appearing in the patient is generally administered. As an example, a daily dose of between 1 and 1000 mg is preferable for an adult.

Examples showing the preparation of compounds of the present invention are described below, though the scope of the present invention is not in any way restricted by these specific example.

Example 1

Ethyl 2-cyano-3-(3,4-dihydroxyphenyl)-2-propenoate (Compound No. 1)

To a solution of 3,4-dihydroxybenzaldehyde (2.0 g, 14.5 mmol) in ethanol (10 ml) were added ethyl cyanoacetate (1.6 g, 14.5 mmol) and piperidine (3 drops) and the resulting reaction was allowed to proceed for 20 hours at room temperature. Water was then added to the reaction mixture, and the precipitated crystalline was filtered off and recrystallized from ethanol-water to yield the titled compound (2.3 g, 9.9 mmol). Alternatively, the reaction may be conducted in a benzene solution under refluxing conditions. Compound Nos. 2 to 6 were provided in a similar manner.

Compound No. 2: 2-cyano-3-(3,4-dihydroxyphenyl)-2-propenoic acid

Compound No. 3: ethyl 2-cyano-3-(3,4,5-trihydroxyphenyl)-2-propenoate

Compound No. 4: 2-cyano-3-(3,4,5-trihydroxyphenyl)-2-propenoic acid

Compound No. 5: methyl 2-cyano-3-(3,4-dihydroxyphenyl)-2-propenoate

Compound No. 6: isopropyl 2-cyano-3-(3,4-dihydroxyphenyl)-2-propenoate

Example 2

(5Z,8Z,11Z,14Z)-eicosatetraenyl-2-cyano-3-(3,4-dihydroxyphenyl)-2-propenoate (Compound No. 7)

Lithium aluminum hydride (90 mg, 2.4 mmol) was suspended in anhydrous tetrahydrofuran (40 ml) and the suspension was cooled to 0 °C under a stream of nitrogen. Then a solution of arachidonic acid (600 mg, 2.0 mmol) in anhydrous tetrahydrofuran (35 ml) was added dropwise to the above suspension under cooling and stirring. The reaction mixture was stirred for 1 hour at 0 °C and then for 1 hour at room temperature. After slow addition of water (0.1 ml), a 10% aqueous solution of sodium hydroxide (0.1 ml) and water (0.3 ml) were further added and the mixture was allowed to stand for 1 hour. Drying the mixture over anhydrous magnesium sulfate followed by concentration in vacuo afforded (5Z,8Z,11Z,14Z)-eicosatetraenol (510 mg, 1.8 mmol).

The above product was dissolved in N,N-dimethylformamide (hereinafter abbreviated as DMF) (10 ml) and cooled to 0 °C. Cyanoacetic acid (150 mg, 1.8 mmol) in DMF (4 ml), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (273 mg, 1.8 mmol) in DMF (2 ml) and 4-dimethylaminopyridine (21 mg, 0.2 mmol) in DMF (2 ml) were successively added to the solution under cooling and stirring and the mixture was stirred for 1 hour at 0 °C and for a further 3 hours at room temperature. Then the reaction mixture was concentrated in vacuo and extracted with diethyl ether after the addition of water. The organic layer was dried over anhydrous magnesium sulfate, concentrated in vacuo and purified by column chromatography to give (5Z,8Z,11Z,14Z)-eicosatetraenyl cyanoacetate (300 mg, 0.8 mmol).

To a benzene solution (10 ml) of the product thus obtained was added 3,4-dihydroxybenzaldehyde (116 mg, 0.8 mmol) in diethyl ether (10 ml) and piperidine (1 drop) and the whole mixture was refluxed for 1.5 hours with continuous removal of water. The reaction mixture was cooled and concentrated in vacuo to obtain the titled compound (240 mg, 0.5 mmol).

Eicosyl 2-cyano-3-(3,4-dihydroxyphenyl)-2-propenoate

(Compound No. 8) was synthesized in a similar manner by replacing arachidonic acid with arachic acid.

Example 3

Phenethyl 2-cyano-3-(3,4-dihydroxyphenyl)-2-propenoate (Compound No. 9)

To a solution of β-phenethyl alcohol (3.6 ml, 30.0 mmol) in DMF (30 ml) was added cyanoacetic acid (2.55 g, 30.0 mmol) in DMF (20 ml) and the whole was cooled to 0 °C. 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimido (4.66 g, 30.0 mmol) in DMF (5 ml) and 4-dimethylaminopyridine (0.37 g, 3.0 mmol) in DMF (10 ml) were then added to the mixture in that order at 0 °C and stirring was continued for 1 hour at this temperature and 18 hours at room temperature. The reaction mixture was concentrated in vacuo and purified by column chromatography to afford phenethyl cyanoacetate (2.49 g, 13.2 mmol).

To a solution of 3,4-dihydroxybenzaldehyde (1.73 g, 12.5 mmol) in DMF (3 ml) was added benzene (30 ml), a portion of the phenyl cyanoacetate obtained above (2.49 g, 13.2 mmol) and benzene (70 ml) in that order and the whole was well mixed. Piperidine (3 drops) was added to the solution and the mixture was refluxed for 2 hours with continuous removal of water using Dean-Stark equipment. Then the reaction mixture was cooled down and concentrated in vacuo. Water was added to the concentrate and the precipitated crystalline was filtered off. The crude product thus obtained was recrystallized from ethanol-water to give the titled compound (3.5 g, 11.3 mmol).

The following compounds were provided in a similar manner by replacing β-phenethyl alcohol with 3-phenyl-1-propanol, 4-phenyl-1-butanol, 5-phenyl-1-pentanol, 4-methoxyphenethyl alcohol, 2-methoxyphenethyl alcohol, 2-(2-thienyl)ethanol, 3-(3-pyridyl)-1-propanol, 3-phenyl-2-propenol, 2-phenoxyethanol, 8-(1-imidazolyl)-1-octanol (e.g. as synthesized according to Japanese Patent Laid-Open No. 112862/1979), respectively:

Phenylpropyl 2-cyano-3-(3,4-dihydroxyphenyl)-2-propenoate (Compound 10)

Phenylbutyl 2-cyano-3-(3,4-dihydroxyphenyl)-2-propenoate (Compound 11)

Phenylpentyl 2-cyano-3-(3,4-dihydroxyphenyl)-2-propenoate (Compound 12)

4-Methoxyphenethyl 2-cyano-3-(3,4-dihydroxyphenyl)-2-propenoate (Compound 13)

2-Methoxyphenethyl 2-cyano-3-(3,4-dihydroxyphenyl)-2-propenoate (Compound 14)

2-(2-Thienyl)ethyl 2-cyano-3-(3,4-dihydroxyphenyl)-2-propenoate (Compound 15)

3-(3-Pyridyl)propyl 2-cyano-3-(3,4-dihydroxyphenyl)-2-propenoate (Compound 16)

3-Phenyl-2-propenyl 2-cyano-3-(3,4-dihydroxyphenyl)-2-propenoate (Compound 17)

2-Phenoxyethyl 2-cyano-3-(3,4-dihydroxyphenyl)-2-propenoate (Compound 18)

8-(1-Imidazolyl)octyl 2-cyano-3-(3,4-dihydroxyphenyl)-2-propenoate (Compound 19)

The compounds with hydroxy substituent(s) on their aromatic rings can be synthesized as follows.

After the protection of phenolic hydroxyl of 4-hydroxyphenethyl alcohol by the formulation of chlorodimethylether, the protected compound was condensed with cyanoacetic acid according to the procedure in Example 1. Further condensation of the product with 3,4-dihydroxybenzaldehyde followed by deprotection in the usual manner provided the following compound.

4-Hydroxyphenethyl 2-cyano-3-(3,4-dihydroxyphenyl)-2-propenoate (Compound 20)

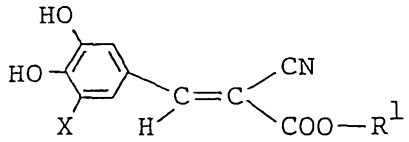
After the reduction of 3,4-dimethoxycinnamic acid to 3-(3,4-dimethoxyphenyl)propanol in the usual manner followed by condensation with cyanoacetic acid according to the procedure in Example 3, the product was demethylated with boron trichloride and condensed with 3,4-dihydroxybenzaldehyde to give the following compound.

3-(3,4-Dihydroxyphenyl)propyl 2-cyano-3-(3,4-dihydroxyphenyl)-2-propenoate (Compound 21)

On the other hand, phenethyl 2-cyano-3-(3,4,5-trihydroxyphenyl)-2-propenoate (Compound No. 22) was obtained by replacing 3,4-dihydroxybenzaldehyde with 3,4,5-trihydroxybenzaldehyde and following the procedure in Example 1.

Physical data in respect of the synthesized compounds Nos. 1 to 22 are shown in Table 1.

Table 1



Compound No.	X	R^1	Appearance	m.p. (°C)	Recrystallized from	IR spectrum (ν cm^{-1}) (nujol)	NMR spectrum (δ ppm) (270 MHz) (CD_3OD)
1	H	C_2H_5	yellow crystalline	169-170	EtOH/water	1700, 2230	1.36(3H,t,J=7Hz), 4.33(2H,q,J=7Hz), 6.86(1H,d,J=9Hz), 7.37(1H,dd,J=2Hz,J=9Hz), 7.64(1H,d,J=2Hz), 8.10(1H,s)
2	H	H	yellow crystalline	220	water	1680, 2230	6.89(1H,d,J=8Hz), 7.38(1H,dd,J=2Hz,J=8Hz), 7.63(1H,d,J=2Hz), 8.10(1H,s)
3	OH	C_2H_5	yellow needle	187	EtOH/water	1695, 2230	1.35(3H,t,J=7Hz), 4.32(2H,q,J=7Hz), 7.13(2H,s), 8.01(1H,s)
4	OH	H	yellow needle	213-215	EtOH/water	1690, 2230	7.10(2H,s), 7.98(1H,s)
5	H	CH_3	yellow needle	200-204	MeOH/water	1695, 2230	3.87(3H,s), 6.87(1H,d,J=8Hz), 7.37(1H,dd,J=2Hz,J=8Hz), 7.64(1H,d,J=2Hz), 8.11(1H,s)

Table 1 (cont'd)

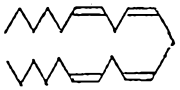
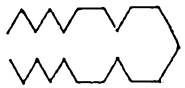
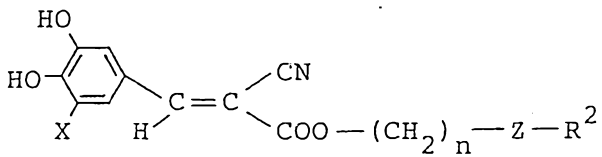
Compound No.	X	R^1	Appearance	m.p. (°C)	Recrystallized from	IR spectrum (ν cm^{-1}) (nujol)	NMR spectrum (δ ppm) (270 MHz) (CD_3OD)
6	H	$CH-CH_3$ | CH_3	pale yellow needle	119-120	iso-propanol	1715, 2240	1.34(6H,d,J=6Hz), 5.1-5.2(1H,m), 6.86(1H,d,J=9Hz), 7.37(1H,dd,J=2Hz,J=9Hz), 7.64(1H,d,J=2Hz), 8.08(1H,s)
7	H		oil	-		1720, 2225 *($CHCl_3$)	0.89(3H,t,J=7Hz), 1.2-2.2(14H,m), 2.8-2.9(6H,m), 4.30(2H,t,J=7Hz), 5.3-5.5(8H,m), 6.95(1H,d,J=8Hz), 7.28(1H,dd,J=2Hz,J=8Hz), 7.85(1H,d,J=2Hz), 8.11(1H,s) *($CDCl_3$)
8	H		yellow crys-talline	123-124	EtOH	1735, 2240	0.89(3H,t,J=7Hz), 1.2-1.5(34H,m), 1.7-1.8(2H,m), 4.27(2H,t,J=6Hz), 6.86(1H,d,J=9Hz), 7.38(1H,dd,J=1Hz,J=9Hz), 7.64(1H,d,J=1Hz), 8.09(1H,s)

Table 1 (cont'd)



Compound No.	X	R^1			Appearance	m.p. (°C)	Recrystallized from	IR spectrum (ν cm^{-1}) (nujol)	NMR spectrum (δ ppm) (270 MHz) (CD_3OD)
		n	Z	R^2					
9	H	2	single bond		yellow crystalline	171-172	EtOH/water	1730 2230	3.03(2H,t,J=7Hz), 4.45 (2H,t,J=7Hz), 6.86(1H,d,J=9Hz), 7.15-7.39(6H,m), 7.63(1H,d,J=2Hz), 8.05(1H,s),
10	H	3	single bond		yellow crystalline	170-173	MeOH/$CHCl_3$	1690 2225	1.92-2.15(2H,m), 2.77 (2H,t,J=7Hz), 4.26(2H,t,J=7Hz), 6.83(1H,d,J=9Hz), 7.10-7.32 (5H,m), 7.36(1H,dd,J=9Hz, 2Hz), 7.65(1H,d,J=2Hz), 8.04(1H,s)
11	H	4	single bond		pale yellow crystalline	149-151	$CHCl_3$	1700 2225	1.60-1.85(4H,m), 2.55-2.77(2H,m), 4.18-4.39(2H,m), 6.85 (1H,d,J=9Hz), 7.08-7.30(5H,m), 7.37(1H,dd,J=9Hz, 2Hz), 7.65(1H,d,J=2Hz), 8.08(1H,s)
12	H	5	single bond		yellow crystalline	144-146	benzene	1690 2225	1.35-1.55(2H,m), 1.55-1.83(4H,m), 2.64(2H,t,J=8Hz), 4.27 (2H,t,J=7Hz), 6.84(1H,d,J=8Hz), 7.05-7.28(5H,m), 7.35(1H,dd,J=9Hz, 2Hz), 7.65(1H,d,J=2Hz), 8.06(1H,s)

Table 1 (cont'd)

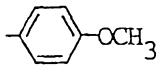
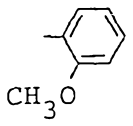
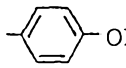
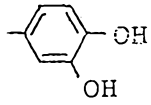
Compound No.	X	R^1			R^2	Appearance	m.p. (°C)	Recrystallized from	IR spectrum (ν cm^{-1}) (nujol)	NMR spectrum (δ ppm) (270 MHz) (CD_3OD)
		n	Z							
13	H	2	single bond			yellow crystalline	194-198	EtOH/water	1680 2210	2.96(2H,t,J=7Hz), 3.76(3H,s), 4.40(2H,t,J=7Hz), 6.84 (1H,d,J=9Hz), 6.86(2H,d,J=9Hz), 7.21(2H,d,J=9Hz), 7.34(1H,dd,J=9Hz, 2Hz), 7.63(1H,d,J=2Hz), 8.04(1H,s)
14	H	2	single bond			yellow crystalline	157-160	EtOH/water	1725 2240	3.04(2H,t,J=7Hz), 3.83(3H,s), 4.42(2H,t,J=7Hz), 6.78-6.97(3H,m), 7.15-7.28(2H,m), 7.34(1H,dd,J=9Hz, 2Hz), 7.62(1H,d,J=2Hz), 8.00(1H,s)
15	H	2	single bond			yellow crystalline	168-170	EtOH/water	1680 2220	3.27(2H,t,J=7Hz), 4.45 2H,t,J=7Hz), 6.83(1H,d,J=9Hz), 6.88-6.99(2H,m), 7.18-7.27(1H,m), 7.35(1H,dd,J=8Hz, 2Hz), 7.65(1H,d,J=2Hz), 8.08(1H,s)
16	H	3	single bond			yellow crystalline	174-177	EtOH/water	1710 2200	2.02-2.16(2H,m), 2.84 (2H,t,J=7Hz), 4.30(2H,t,J=7Hz), 6.84(1H,d,J=8Hz), 7.31-7.48 (2H,m), 7.65(1H,d,J=1Hz), 7.77(1H,d,J=8Hz), 8.04(1H,s), 8.33-8.40(1H,m), 8.45(1H,d,J=1Hz)
17	H	1	-CH=CH-			yellow crystalline	171-174	EtOH/water	1695 2200	4.93(2H,d,J=6Hz) 6.33-6.48(1H,m), 6.69-6.88(2H,m), 7.20-7.48(6H,m), 7.67(1H,d,J=2Hz), 8.13(1H,s)

Table 1 (cont'd)

Compound No.	X	R^1			Appearance	m.p. (°C)	Recrystallized from	IR spectrum (ν cm^{-1}) (nujol)	NMR spectrum (δ ppm) (270 MHz) (CD_3OD)
		n	Z	R^2					
18	H	2	-O-		yellow crystalline	186-189	EtOH/water	1720 2220	4.30(2H,t,J=5Hz), 4.60 (2H,t,J=5Hz), 6.84(1H,d,J=9Hz), 6.85-6.98(3H,m),7.20-7.33(2H,m), 7.36(1H,dd,J=9Hz, 2Hz), 7.65(1H,d,J=2Hz), 8.09(1H,s)
19	H	8	single bond		yellow crystalline	184-186	MeOH	1720 2210	1.18-1.52(8H,m), 1.61-1.87(4H,m), 4.01(2H,t,J=7Hz), 4.26(2H,t,J=7Hz), 6.87(1H,d,J=9Hz), 6.95(1H,brs), 7.11(1H,brs), 7.38(1H,d,J=7Hz), 7.65(2H,brs), 8.09(1H,s)
20	H	2	single bond		yellow crystalline	186-189	ethyl acetate/hexane	1725 2225	2.93(2H,t,J=7Hz), 4.39 (2H,t,J=7Hz), 6.72(2H,d,J=9Hz), 6.85(1H,d,J=8Hz), 7.11(2H,d, J=9Hz), 7.35(1H,dd,J=8Hz, 2Hz), 7.64(1H,d,J=2Hz), 8.05(1H,s)
21	H	3	single bond		yellow crystalline	172-176	EtOH/water	1695 2220	1.82-2.07(2H,m), 2.62 (2H,t,J=6Hz), 4.24(2H,t,J=6Hz), 6.53(1H,dd,J=9Hz, 2Hz), 6.58-6.72 (2H,m), 6.87(1H,d,J=9Hz), 7.37(1H,dd,J=9Hz, 2Hz), 7.66(1H,d,J=2Hz), 8.06(1H,s)
22	OH	2	single bond		yellow crystalline	170-172	EtOH/water	1690 2220	3.03(2H,t,J=7Hz), 4.44(2H,t,J=7Hz), 7.12(2H,s) 7.15-7.38(5H,m), 7.99(1H,s)

Examples of formulation are shown below.

Formulation 1 (capsules)

Compound No. 1	10 g
Lactose	30 g
Corn starch	30 g
Crystallinecellulose	28 g
Magnesium stearate	2 g
Total	100 g

The above mixture was formed into granules in the usual manner and packed in 200 hard gelatin capsules. Each capsule thus obtained contains 50 mg of the active ingredient.

Formulation 2 (tablets)

Compound No. 9	20 g
Lactose	40 g
Corn starch	20 g
Hydroxypropylcellulose	18 g
Talc	2 g
Total	100 g

The above mixture was blended in a mixer and formed into 200 tablets of 500 mg each. Each tablet thus obtained contains 200 mg of the active ingredient.

Examples of Pharmacological Tests

The 12-lipoxygenase inhibitory activity of the compounds of the present invention was measured by conducting the three types of pharmacological tests described below.

Method 1

Citrated blood was collected from the abdominal aorta of a rat. Washed platelet was prepared in the usual way, diluted with phosphate buffer (pH = 8.0) containing 0.45% NaCl and used as the enzyme solution. To the solution were added the sample compound (10 μM), [^{14}C]arachidonic acid (0.2 μCi) and arachidonic acid (3 μg) and the mixture was reacted for 3 to 5 minutes at 37°C. The reaction mixture was developed by thin layer chromatography and 12-HETE was

identified by a radio chromato scanner. The reduction in 12-HETE production which resulted was used as the index of 12-lipoxygenase inhibitory activity.

Method 2

Citrated blood was collected from the heart of a rat and platelet-rich plasma was prepared in the usual way. The plasma was washed twice with isotonic buffer solution (A) [134 mM NaCl, 5 mM D-glucose, 1 mM EDTA, 1 mM EGTA, 15 mM Tris-HCl (pH = 7.4)], frozen and stored at -80 °C. It was thawed before use, ultrasonified in ice water and used as the enzyme solution. To the isotonic buffer solution (A) were added 1 mM GSH, the sample compound (final concentration: 1 μM) and the enzyme solution (300 to 500 μg protein) and the mixture was pre-incubated for 5 minutes at 37 °C; then [^{14}C]arachidonic acid (0.05 μCi) (final concentration: 4.3 μM) was further added and reacted for 5 minutes. After the reaction had been quenched, the reaction mixture was developed on a silica gel thin layer plate and 12-HETE was identified by autoradiography. The reduction in 12-HETE production was used as the index of 12-lipoxygenase inhibitory activity.

Method 3

The platelet-rich plasma obtained in the same way as described in Method 2 was washed successively with isotonic buffer solution (A) and isotonic buffer solution (B) [134 mM NaCl, 5 mM D-glucose, 0.1 mM DETA, 0.1 mM EGTA, 15 mM Tris-HCl (pH = 7.4)] in that order, and suspended in a hypotonic buffer solution (C) [25 mM Tris-HCl (pH = 7.7)]. The suspension was treated by a "freeze-thaw" procedure three times and centrifuged at 10500 x g for 60 minutes. An aliquot of the supernatant (platelet soluble fraction) was used as the enzyme solution. To the hypotonic buffer solution (C) were added 1 mM GSH, [^{14}C]arachidonic acid (0.025 μCi) (final concentration: 40 μM), the sample compound (final concentration: 10 μM) and the enzyme solution (50 μg protein) and the mixture was reacted for 5 minutes at 37 °C. The index of 12-lipoxygenase inhibitory activity was obtained in the same way as described in Method 2.

Results

The production indexes of 12-HETE under the inhibitory influence of the compounds of the present invention are shown in Table 2, where the production amount of the control experiment (in the absence of the sample compound) is 100%.

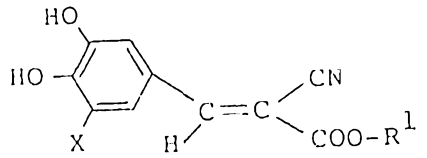
Table 2

Compound No.	Method 1 (%)	Method 2 (%)	Method 3 (%)
1	38.1		
5	36.0		
6	31.4		
9		10.0	25.1
10		12.1	
11		14.2	
13			19.5
14			19.1
15		8.7	
17		13.2	
18		8.5	
Baicalein (for comparison)	27.0	9.8	22.7

Since caffeic acid derivatives of the present invention exhibit 12-lipoxygenase inhibitory activity, they are useful for the prevention and remedy of circulatory diseases such as arteriosclerosis. The caffeic acid derivatives of the present invention can be easily made by synthesis.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A caffeic acid derivative of the general formula (I) and pharmaceutically permissible salts thereof:



wherein X is hydrogen atom or hydroxy; R^1 is hydrogen atom, a straight or branched alkyl or alkenyl having 1 to 20 carbon atoms, or a group of the formula:

$$-(CH_2)_n-Z-R^2$$

wherein n is an integer of 1 to 10; Z is oxygen atom, vinylene residue or a single bond, and R^2 is a phenyl which may be substituted with up to 3 substituents selected from hydroxy, a lower alkoxy or a halogen atom or R^2 is a heterocyclic residue; provided that X and R^1 each cannot be a hydrogen atom simultaneously.

2. A compound as claimed in Claim 1, wherein R^1 in the general formula is hydrogen atom or a straight or branched alkyl or alkenyl having 1 to 20 carbon atoms.

3. A compound as claimed in Claim 1, wherein R^1 in the general formula is a group of the formula:

$$-(CH_2)_n-Z-R^2$$

wherein n is an integer of 1 to 10; Z is oxygen atom, the vinylene residue or a single bond, and R^2 is a phenyl which may be substituted with up to 3 substituents selected from hydroxy, a lower alkoxy or a halogen atom or R^2 is a heterocyclic residue; provided that X and R^1 each cannot be a hydrogen atom simultaneously.

4. A pharmaceutical composition used as a remedy for circulatory diseases which comprises a pharmaceutically effective amount of a compound of Claim 1.

5. A compound according to any one of claims 1 to 3 substantially as herein described with reference to any one of the Examples.