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Office européen des brevets

Publication number:

0 089 229
A2

(12)

EUROPEAN PATENT APPLICATION

(21) Application number: 83301424.4

(51) Int. Cl.³: **C 07 D 493/14**

(22) Date of filing: 15.03.83

A 61 K 31/36

//(C07D493/14, 317/00, 311/00,
307/00)

(30) Priority: 15.03.82 US 357805
15.03.82 US 358191

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(43) Date of publication of application:
21.09.83 Bulletin 83/38

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(84) Designated Contracting States:
AT BE CH DE FR GB IT LI LU NL SE

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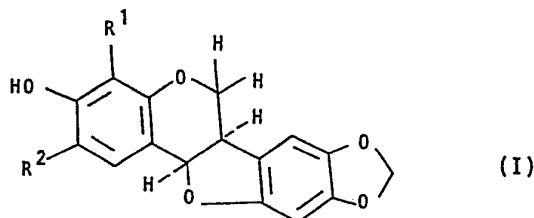
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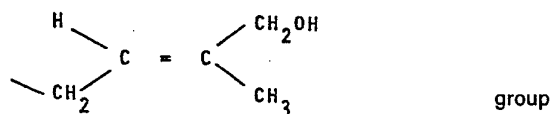
(54) **Physiologically active pterocarpan compounds, methods for the isolation thereof and therapeutically active compositions containing them.**

(57) The invention relates to physiologically active pterocarpan-compounds and methods for the isolation thereof. These compounds have the formula I

are effective against the toxic venoms of poisonous snakes, spiders and other insects, and against *E. coli* endotoxins. The invention further relates to therapeutically active compositions containing the above pterocarpan compounds.



wherein R¹ represents a hydrogen atom and R² represents a -CH₂CH₂-CH(CH₃)-CH₂OH group, or R¹ represents a



and R² represents a hydrogen atom, together with all optical isomers and racemic mixtures thereof. These compounds

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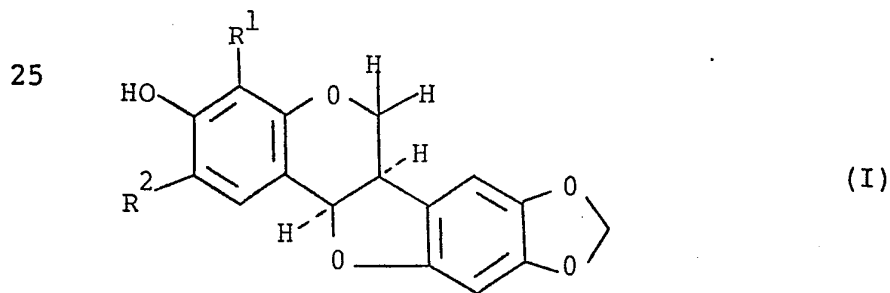
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"Physiologically active pterocarpan-compounds,
methods for the isolation thereof and
therapeutically active compositions
containing them"

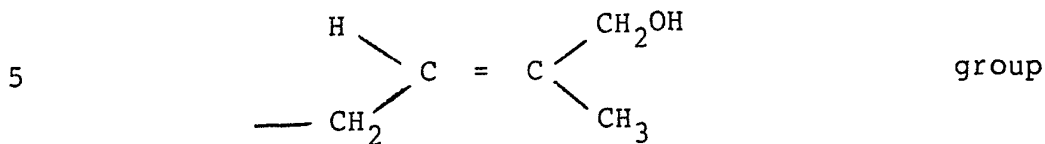
This invention relates to physiologically active pterocarpan-compounds, methods for the isolation thereof and therapeutically active compositions containing them. More particularly it relates to products of
 5 manufacture which are substantially pure crystalline pterocarpan compounds which have been isolated from the crude extracts of natural products and to methods for isolation of the compounds.

It is known that the aqueous alcoholic extract
 10 of the root of the South American cabeza de negra tree has been available to plantation workers in the upper Amazon jungle as an oral antidote against snake and spider venoms. About ten varieties of the species cabeza de negra are known in South America.
 15 Neither the nature of the active components nor studies of the side effects, pharmacological activity, stability and the like are known to have been published on the compounds of the invention. Pterocarpan possessing antimicrobial properties have been identified in
 20 the literature, see Heterocycles, Vol. 15, 1163 [1981].

In one aspect, the invention relates to pterocarpan compounds having the formula (I)



wherein R¹ represents a hydrogen atom and R² represents a -CH₂CH₂-CH(CH₃)-CH₂OH group, or R¹ represents a



and R² represents a hydrogen atom, together with all optical isomers and racemic mixtures thereof.

10 We have been able to isolate such compounds in substantially pure crystalline form. For convenience, the compounds of the above structure will be referred to hereinafter as cabenegrin I [3-hydroxy-4-(3-hydroxy-methyl-butene-2)-8,9-methylenedioxy-pterocarpan]

15 and cabenegrin II [2-(3-hydroxymethyl-butyl)-3-hydroxy-8,9-methylenedioxy-pterocarpan], respectively. The invention also relates to a step-wise method employed to recover and isolate the essentially pure - 95-100% - crystalline products referred to as cabenegrin

20 I and cabenegrin II.

The compounds cabenegrin I and cabenegrin II have pharmaceutical utility in the treatment of mammals, including man, that have been envenomated by poisonous snakes and insects. These compounds have been found

25 to acts as potent antidotes against effects of snake and insect toxins. The compounds of the invention are also useful in treating the effects of other known organic toxins such as E. coli endotoxin and that produced by Clostridium botulinum, commonly

30 referred to as botulism on food poisoning. Pharmaceutical utility is generally indicated for the treatment of the effects in mammals of toxins produced by pathogenic bacteria (endotoxins and exotoxins) which attack the nervous system of the victim and in particular

35 where paralysis of the respiratory system is manifested. Treatment against the effects of cardio-vascular toxins is also indicated.

The compounds can be administered orally e.g. in liquid form as a suspension or solubilized in a compatible pharmaceutical carrier. Pure crystalline material can be administered orally in capsule or tablet form
5 when compounded with suitable pharmaceutical carriers. Alternatively, either of the compounds cabenegrin I or II can be dissolved in a suitable liquid pharmaceutical carrier and the solution administered intravenously by syringe, or by catheter where monitoring equipment
10 is available to determine the effects of the therapy. If desired, the compounds of the invention can be mixed together in various proportions, and the mixture prepared as described above with suitable pharmaceutical carriers.

15 A crude extract from which the compounds of the invention may be obtained may be prepared as follows:

Approximately $\frac{1}{2}$ kilo of the washed root of the cabeca de negra tree is chopped into small pieces
20 which may be macerated, pulverized or otherwise treated to break down the fibrous structure. This step can be accomplished in a blender or laboratory homogenizer. Roots evidencing mold or fungus should not be used. The pulverized root is placed in a large glass beaker
25 or other suitable vessel which may be covered and subsequently stirred. A sufficient quantity of the mixture of water and aliphatic alcohol having 1-4 carbon atoms, preferably ethanol:water 77:23 is added to cover the pulverized root and briskly stirred for
30 a few minutes. The vessel containing the aqueous ethanol and root is allowed to stand at ambient temperatures for at least 48 hours, with occasional stirring. At the end of this period, the aqueous alcoholic solution comprising the crude extract is separated
35 from the root by any convenient means, for example, by pouring through medium filter paper. The pulverized root is discarded. The filtered solution is the color of strong tea.

The following procedure may then be employed to isolate the active compounds from the crude extract as prepared above.

The aqueous ethanol crude extract (135 ml) was concentrated by gently warming under vacuum to obtain 1.2 grams of a brown oily residue. This concentrated material was treated with 50% aqueous methanol and the solution was extracted by vigorous shaking with an aliphatic hydrocarbon having 5-8 carbon atoms, preferably with hexane. The hexane layer was separated and discarded. The water layer was extracted with ether by vigorous shaking. The ether layer and the water layer was separated, the ether layer (640 milligrams) being set aside. The water layer was extracted with n-butyl alcohol by vigorous shaking. The n-butyl alcohol layer was separated and set aside for a possible investigation, and the water layer was discarded.

The ether layer extracted above is separated using well-known chromatographic methods. Preferably, the ether layer extracted above is subjected in a first step to high pressure liquid chromatography (HPLC) on Sephadex LH-20 and as a second step on silica gel using aqueous methanol as the eluting solvent. This procedure results in two fractions and the first is further separated by HPLC employing Partisil-10 eluted with 3% methanol in methylene chloride to yield pure solid compounds.

The compound identified as cabenegrin I is recovered as a white crystalline material in a yield of 44 mg. A sharp melting point of 167-168°C is obtained and analysis shows the composition to be $C_{21}H_{20}O_6$. The Rf value of this compound on thin layer chromatography employing silica gel CO/Kieselguhr F-254 and benzene/ethyl acetate/methanol (15/4/1) was 0.53.

The U.V., C.D., and I.R. of compound I are as follows:

UV (in MeOH): 209 nm (ϵ 75,000), 233 nm shoulder,
 (ϵ 24,000), 309 nm (ϵ 13,000). CD (in MeOH): 213 nm
 ($\Delta\epsilon$ -25.38), 220 nm ($\Delta\epsilon$ -2.00), 238 nm ($\Delta\epsilon$ -9.84);
 302 nm ($\Delta\epsilon$ +3.15). IR (in CHCl_3): 3550 cm^{-1} , 1600 cm^{-1} ,
 5 1113 cm^{-1} , 925 cm^{-1} .

In addition to these spectral data, the following
 $^1\text{H-NMR}$, $^{13}\text{C-NMR}$, and MS (EI) data were measured concerning
 cabenegrin I:

$^1\text{H-NMR}$: 1.83 (3H, d, 1 Hz, 4'- CH_3);
 10 3.40 (2H, br, d, 7 Hz, $\overline{\text{C}} 1'$);
 3.40 (2H, ddd, 7, 7.5 Hz, C 6a);
 3.61 (2H, d, d, 11.7 Hz, c 6 β);
 3.90 (2H, $-\text{OCH}_2\text{O}$);
 3.99 (2H, br, s, $-\text{CH}_2-\text{OH}$);
 15 4.28 (1H, dd, 11.5 Hz, C 6 α);
 5.45 (1H, br, t, 7 Hz, C 2');
 5.45 (1H, d, 7 Hz, C 11a);
 6.41 (1H, s, C 10);
 6.52 (1H, d, 9 Hz, C 2);
 20 6.70 (1H, s, C 7);
 7.23 (1H, d, 9 Hz, C 1) ppm.

$^{13}\text{C-NMR}$: 13.74 (4'- CH_3); 21.88 (C 1'); 40.16 (C 6a);
 25 66.72 (C 6); 68.72 ($-\text{CH}_2-\text{OH}$); 79.10 (C 11a);
 93.77 (C 10); 104.73 (C 7); 109.6 (C 2);
 112.63 (C 4 or C 1a); 115.01 (C 4 or C 1a);
 118.04 (C 6b); 123.49 (C 2'); 129.15 (C 1);
 136.07 (C 3'); 141.67 (C 8); 148.06 (C 9);
 154.20 (C 10a or C 4a); 154.20 (C 10a or C 4a);
 30 155.08 (C 3) ppm.

MS (18 eV):

M^+ = 368, 50%
 m/e = 350, 100%; 335, 95%; 335, 95%;
 35 176, 65%; 161, 90%.

The second sample of material recovered from
 the HPLC described above comprised an oily mixture
 of approximately 10 mg. This second active fraction,

which had been obtained from the HPLC of the ether layer described above, is treated as follows to obtain the compound cabenegrin II. The mixture was subjected to further HPLC employing μ -Bondapack C18 and methanol/ 5 acetonitrile/H₂O/n-PrOH (71/71/59/2). The compound (II) was obtained in essentially pure - 95-100% - crystalline form in a yield of about 1 mg. The structure of this compound is based on the following physical constants, and analysis (MS) shows the composition to be C₂₁H₂₂O₆.

10

¹H-NMR: 0.69 (3H, d, 5.8 Hz, 3'-CH₃);
2.50 (2H, m, C 1');
2.67 (2H, m, C 1');
2.94 (1H, ddd, 9.1, 6.3, 4.9Hz, C 6a);
15 3.10 (2H, dd, 8.8, 2.7 Hz, -CH₂-OH);
3.18 (2H, dd, 8.8, 4.4 Hz, -CH₂-OH);
3.50 (1H, dd, 9.1, 9.1 Hz, C 6β);
3.86 (1H, dd, 9.1, 4.9 Hz, C 6α);
5.27 (1H, d, 6.3 Hz, C 11a);
20 5.30 (2H, -OCH₂O-);
5.33 (2H, -OCH₂O-);
6.36 (1H, C 10);
6.52 (1H, C 4);
6.53 (1H, C 7);
25 7.08 (1H, C 1) ppm.

UV (in MeOH): 204 nm (ε 116,000), 230 nm (ε 8,000),
292 nm (ε 9,400), 308 nm (ε 11,800).

30 CD (in MeOH): 237 nm (Δε -6.68), 280 nm (Δε -0.46),
299 nm (Δε -1.72).

The structure of this compound is shown by NMR data to be a 3:1 mixture of epimers at C-3' and 35 the invention includes the individual optical isomers together with racemic mixtures within its scope. The structures of both compounds cabenegrin I and

cabenegrin II have been confirmed by synthesis of their respective racemates.

The new compounds produced by the method of the invention are suitable for making therapeutically active compositions, which are antidotes for treating the effects of poisonous snake and insect bites in mammals, including man.

They are also suitable for the treatment of pathogenic bacterial toxins such as E. coli endotoxins, botulism and others which exhibit central nervous system effects and related respiratory paralysis, and to the treatment of the effects of cardiovascular toxins on mammals.

As for the new compounds obtained by the above extraction methods, the presence of physiologically active compounds was determined by in vivo tests employing mice. Test animals were Swiss Webster white mice, mixed sexes, weighing from 25-30 grams. Each group of test animals was envenomated with two and one-half times the lethal dose of snake venom from the Fer de Lance (Bothrops atrox) by intraperitoneal injection. In the absence of treatment, envenomated animals succumbed within a few minutes.

Concentrates of compounds of the invention obtained from each of the fractions of the above extraction scheme were tested for antidotal activity by injecting the mice immediately after envenomation with an aqueous ethanol solution (77.23) of the material isolated from each fractions. Each animal was treated with 0.25 ml of the respective solutions. On the basis of this protocol, the minimum dosage for survival against the Fer de Lance venom was 2.8 mg/kg of cabenegrin I and 2.0 mg/kg of cabenegrin II.

Toxicological studies indicate that administration of aqueous ethanolic solutions of cabenegrin I and cabenegrin II to healthy test animals produce no significant changes in vital physiological functions. Administration can be by intravenous or intramuscular

injection, or orally via a stomach tube. No significant change is noted in arterial blood pressure, heart rate, respiration, EKG or central venous pressure at any time following administration of compounds
5 I and II to normal healthy (i.e. non-venomated) animals.

Administration does not significantly alter resting action potentials, end plate potentials, nerve impulse transmission, neuro-muscular function
10 or brain wave activity in experimental animals.

1. Antidotal effects against snake venom in dogs.

Envenomation by a lethal dose of snake venom, such as Bothrops atrox, Crotalus adamanteus, or Crotalus atrox, produces a precipitous fall in arterial
15 blood pressure, a decrease of heart rate and an elevation in central venous pressure. This is followed by partial recovery of these parameters and then by a complete respiratory and cardiovascular collapse. Death appears to be due to a combination of peripheral
20 vascular collapse and to an interruption in the normal respiratory mechanism. In addition, there appears to be some action of these venoms on the central nervous system of the experimental animals. This CNS effect is exhibited by a decrease in both the
25 alpha and the beta rhythm of the brain (EEG). This change is also associated with a decrease in impulse transmission over the motor nerves and progressive blockage of the neuromuscular apparatus which is similar to that produced by curare. Venoms had no
30 effect on muscle response to direct stimulation.

A series of seven adult beagle dogs are used to study the effectiveness of cabenegrin I and II against the venoms. The dogs are anaesthetized with Na pentobarbital (30 mg/kg) and monitored for changes
35 in arterial blood pressure, heart rate, electrocardiogram and respiration. Lethal doses (five to ten times of LD₅₀) of lyophilized reconstituted Bothrops atrox

(Fer de Lance) (2.5 mg - 5.0 mg/kg) or (10 mg/kg) South American rattle snake venom are administered.

Within 15 minutes following envenomation, marked decreases in heart rate and blood pressure are consistently
5 noted. At from 15 to 30 minutes, respiration likewise decreases from an average of 20 per minute to 5 per minute. Treatment is initiated when severe cardiovascular embarrassment and apparent respiratory difficulties are observed, usually at from 15-30 minutes
10 following envenomations.

A solution of cabenegrin I is prepared by dissolving 33 mg of the crystalline material of compound in 100 ml of aqueous ethanol (25:75). Similarly, a solution of 24 mg of cabenegrin II in 100 ml of aqueous
15 ethanol (15:75) is prepared. Doses are prepared for administration by stirring 5 ml of each of the respective alcoholic solutions of the compounds of the invention into 50 ml of water.

Administration of the respective solutions
20 is through a tube placed and advanced into the stomach of the dog. Treatment is as follows: No immediate response is noted following therapy. Blood pressure, heart rate and respiration all remain extremely low. At approximately 30 minutes following the first dose
25 of the respective alcoholic solution of the compound of the invention, a slow gradual improvement of breathing occurs followed by partial restoration of heart rate and blood pressure. Continuous therapy is provided at 30 minute intervals in 50 ml water until all monitored
30 vital signs return to within 10% of control. The effective dose range is between 10 to 20 ml of antivenom extract per animal. From two to four doses are required. After observation for 8-10 hours the animals are placed in a holding cage with food and water. At
35 24 hours, all 7 dogs show signs of depressed activity. At 72 hours, all dogs are taking food and water. No additional therapy is required.

2. Activity of cabenegrin I and II against E. coli endotoxin

Three adult beagle dogs are used to demonstrate the effectiveness of the cabenegrin I and II in treating
5 shock caused by E. coli endotoxin. The dogs are anaesthetized with Na pentobarbital (30 mg/kg) and monitored for changes in arterial blood pressure, heart rate, EKG and respiration. Lethal doses (1 mg/kg) of E. coli endotoxin are injected i.v. into a catheter
10 placed in the vein of the hind limb of the dogs. In the first experiment no antidotal therapy was initiated and the animal expired 2 hours after injection. In the three additional cases antidotal therapy is initiated at the time when severe cardiovascular
15 collapse and respiratory difficulties appear. These usually occur within about 1½ hours after the injection of the toxin.

Solutions of cabenegrin I and II, prepared as described above, are administered by stomach tube
20 in a single dose of 10 ml in 100 ml of water to each of the dogs. The animals so treated survive and resume normal activity.

3. Isolated heart (Langendorff) preparation

25 A series of 2 dog heart preparations are tested to measure the antivenom effect on coronary blood flow, heart rate, EKG, force of ventricular contraction and coronary vascular resistance when cabenegrin I and II are given either before or after lethal
30 venom challenge. It is observed that treatment appears capable of overcoming the toxic effects of the venom on cardiovascular functions. These effects are a decrease of the force of contraction and heart rate. Coronary vascular resistance also increases progressively
35 following the administration of venom. When solutions containing 0.05 mg/ml of either of the compounds of the invention are injected directly into the circulation prior to tropical rattle snake venom challenge,

no detrimental effect on the heart is observed. Rather, the force of contraction and coronary blood flow increases by about 15 to 20%.

When either of the compounds is given following
5 lethal challenge of tropical rattle snake venom, the antidote restores force of contraction and heart rate to normal levels and reverses the minor arrhythmias caused by envenomation.

4. Neurophysiological function

10 Three dogs and one cat are tested for the antidotal effect on neuromuscular function, action potential and brain wave activity following envenomation with lethal doses of Fer de Lance venom.

Snake venom decreases both brain wave activity
15 and nerve impulse transmission. These are restored to nearly, if not completely, normal levels by the administration of cabenegrin I and II. Action potentials and neuromuscular function remain depressed for approximately 30 to 60 minutes after treatment with each
20 of the compounds. This is followed by a slow, gradual return to control levels after from 12 to 24 hours.

In certain experiments in which complete neuromuscular blockage occurs and the animals are no longer capable of spontaneous respiration, artificial ventilation
25 is required until the action of the compound has manifested itself. This may occur after envenomation, but once stabilized, the animals are capable of spontaneous breathing and no further therapy is required.

Cortical electrical activity is markedly (25-30%)
30 depressed by the venom. These changes are restored to normal by the administration of the compounds cabenegrin I and II. Following treatment no further changes are noted.

Results of these studies indicate that oral
35 or i.v. doses of cabenegrin I and II are capable of treating conditions clinically thought of as being either cardiotoxic and/or neurotoxic in nature with no inherent observable side effects.

As mentioned above, the new compounds of the method of the invention are suitable for the preparation of pharmaceutical compositions, preferably for oral administration, or for parenteral injections.

5 Suitable pharmaceutical carriers for oral administration include liquids which are inert to the gastric mucosa. Liquid carriers can be of the type in which a stable suspension of compounds of the invention can be prepared. Alternatively the liquid carrier
10 can be a solvent for the cabenegrin I or II. In the latter case, the liquid pharmaceutical carrier solution can be prepared for either oral administration, or for parenteral injection.

Novel compositions for oral administration
15 can also be prepared by blending cabenegrin I and II with appropriate dry pharmaceutical carriers known to the art. These dry compositions can be put into any suitable dosage form for ingestion including pills, tablets and capsules. Micro-encapsulation
20 techniques can be employed to provide a sustained release of the desired dosage if the particular condition of the subject indicates this form of therapy.

In many instances, either the nature of the poisonous toxin, or the type of deteriorating condition
25 of the subject will necessitate that a liquid dosage be administered to insure a prompt initiation of the therapeutic effects of the compounds. Effective treatment of animals or of subjects that are unconscious or whose vital functions are in an advanced state of
30 deterioration will require administration of oral doses via stomach tube or intravenous injection by syringe or catheter.

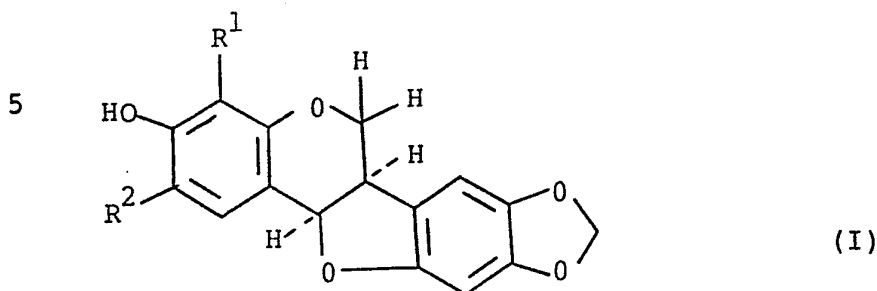
A pharmaceutical composition was prepared by blending the following materials in the specified
35 proportions by weight:

	Cabenegrin I	20
	Starch	15.0
	Magnesium stearate	2.0
	Sodium benzoate	6.0
5	Benzalkonium chloride	2.0

After the dry composition was thoroughly blended tablets were prepared from the mixture. Each tablet was formed so that it contained 100 mg of cabenegrin I. Similarly, tablets were prepared using the same mixture for the pharmaceutical carrier and the same proportion of cabenegrin II was substituted for the compound, with each tablet containing 70 mg of cabenegrin I.

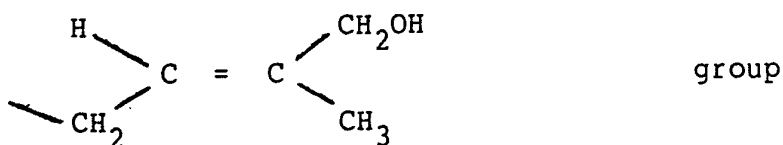
Claims:

1. A compound of the formula (I)



10

wherein R^1 represents a hydrogen atom and R^2 represents
 15 a $-\text{CH}_2\text{CH}_2-\text{CH}(\text{CH}_3)-\text{CH}_2\text{OH}$ group, or R^1 represents a



20

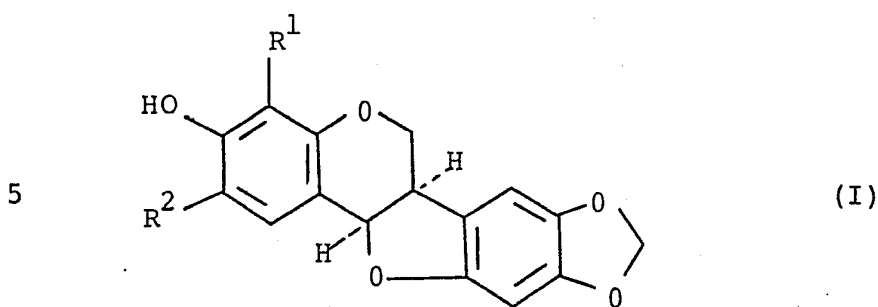
and R^2 represents a hydrogen atom, together with
 all optical isomers and racemic mixtures thereof.

2. A mixture of compounds of the formula (I) as
 defined in claim 1 wherein R^2 represents a 3-hydroxymethyl-
 25 but-1-yl group which comprises a 3:1 mixture of epimers
 at C-3' of the butyl group.

3. A compound as claimed in claim 1 or mixture
 as claimed in claim 2 in crystalline form.

4. A compound or mixture as claimed in any of
 30 claims 1 to 3 substantially as hereinbefore described
 with reference to any of the n.m.r., u.v., C.D, and
 I.R. and mass spectral data.

5. A process for the isolation of a compound
 of the formula (I)



10

wherein R¹ and R² are as defined in claim 1 which
15 comprises the steps of

- a) preparing a crude aqueous alcoholic extract
from the comminuted root of the cabeza de negra
tree;
- b) concentrating said extract to provide an oily
20 residue;
- c) treating said oily residue with aqueous methanol
and thereafter extracting with an aliphatic
hydrocarbon having 5-8 carbon atoms;
- d) extracting the remaining aqueous layer with
25 ether; and
- e) separating the ether layer which results by
chromatography to obtain a compound of formula
(I).

6. A process as claimed in claim 5 which comprises
30 extracting the oily residue obtained in step (c)
with hexane.

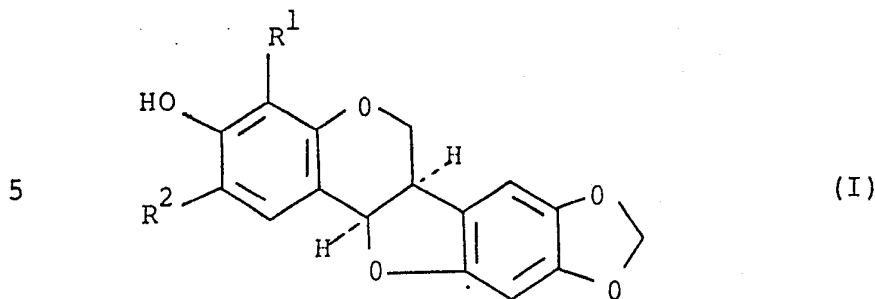
7. A process as claimed in claim 5 or claim 6
which comprises subjecting the ether layer resulting
from step (e) to high pressure liquid chromatography,
35 eluting with methanol, and separating a first fraction
and a second fraction and recovering from said first
fraction a compound of formula (I) wherein R² is
hydrogen and R¹ is a 3-hydroxymethylbut-2-en-1-yl

group and from said second fraction a compound of formula (I) wherein R^1 is hydrogen and R^2 is a 3-hydroxymethyl-but-1-yl group.

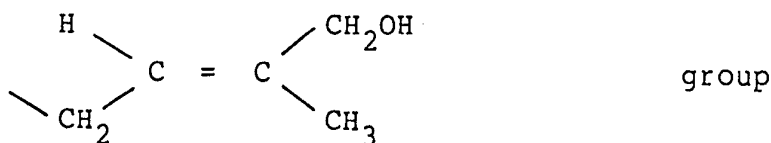
8. A process as claimed in claim 7 wherein further
5 purification is achieved either by subjecting the first fraction to high pressure liquid chromatography and eluting with methanol and methylene chloride and/or subjecting said second fraction to high pressure
10 liquid chromatography and eluting with methanol, acetonitrile, water and n-propylalcohol.
9. A therapeutic composition comprising a compound of formula (I) or mixture of one or more thereof as defined in any of claims 1 to 4 in admixture with a pharmaceutically acceptable carrier, diluent or excipient.
- 15 10. A composition as claimed in claim 9 wherein the pharmaceutical carrier is a liquid and the composition is suitable either for oral or parenteral administration.

Additional set of claims under Art. 167 (2)(a)
for Austria

1. A process for the isolation of a compound of the formula (I)



wherein R¹ represents a hydrogen atom and R² represents a -CH₂CH₂-CH(CH₃)-CH₂OH group, or R¹ represents a



and R² represents a hydrogen atom, together with
10 all optical isomers and racemic mixtures thereof,
which comprises the steps of

- a) preparing a crude aqueous alcoholic extract from the comminuted root of the cabeza de negra tree;
- 15 b) concentrating said extract to provide an oily residue;
- c) treating said oily residue with aqueous methanol and thereafter extracting with an aliphatic hydrocarbon having 5-8 carbon atoms;
- 20 d) extracting the remaining aqueous layer with ether; and
- e) separating the ether layer which results by chromatography to obtain a compound of formula (I).

2. A process as claimed in claim 1 which comprises extracting the oily residue obtained in step (c) with hexane.
3. A process as claimed in claim 1 or claim 2
5 which comprises subjecting the ether layer resulting from step (e) to high pressure liquid chromatography, eluting with methanol, and separating a first fraction and a second fraction and recovering from said first fraction a compound of formula (I) wherein R^2 is
10 hydrogen and R^1 is a 3-hydroxymethylbut-2-en-1-yl group and from said second fraction a compound of formula (I) wherein R^1 is hydrogen and R^2 is a 3-hydroxymethyl-but-1-yl group.
4. A process as claimed in claim 3 wherein further
15 purification is achieved either by subjecting the first fraction to high pressure liquid chromatography and eluting with methanol and methylene chloride and/or subjecting said second fraction to high pressure liquid chromatography and eluting with methanol,
20 acetonitrile, water and n-propylalcohol.
5. A process as claimed in any of claims 1 to 4 wherein the compound of formula (I) obtained is a compound wherein R^2 represents a 3-hydroxymethyl-but-1-yl group which comprises a 3:1 mixture of epimers at C-3' of the butyl group.
- 25 6. A process as claimed in any of claims 1 to 5 wherein the compound of formula (I) is obtained in crystalline form.
7. A process as claimed in any of claims 1 to 6 wherein the compound of formula (I) obtained is a compound substantially as hereinbefore described with reference to any of
30 the n.m.r., u.v., C.D, and I.R. and mass spectral data.
8. A process for the preparation of a therapeutic composition which comprises admixing a compound of formula (I) or mixture of one or more thereof with a pharmaceutically acceptable carrier, diluent or excipient.
- 35 9. A process as claimed in claim 8 wherein the pharmaceutical carrier is a liquid and the composition thus

prepared is suitable either for oral or parenteral administration.