Koji NAKANISHI, *et al.*
Cabenegrin Resurrection

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Amazing 'Tree of Life' Proves That Reincarnation Is Real

by Frank Kendal

Men and women are being brought back from the dead by the mysterious extract from a tree of life that grows only in the most remote regions of the Amazon.

And amazing stories they tell prove conclusively that there is life after death.

Staggering reports of the miraculous recoveries of men, women and children and their accounts of reincarnation have been documented by excited research scientists who've carried out extensive tests throughout South America.

Now they say they're almost at the stage where they'll be able to make the tree extract available to all mankind.

"The potential application of the compound is mind-boggling", says forensic biochemist Dr Howard Wynn-Hughes.

"It could add a whole new dimension to the way we live our lives."

Wynn-Hughes and his colleagues were astounded at the first-hand stories they heard after hacking their way through dense brush to a remote clinic in Paraguay.

One man who'd been brought back from the dead said the extract helped him to recall that, in an earlier existence, he'd been a conquistador from Spain, a captain named Don Aldro Valdez.

Later, Wynn-Hughes checked his story with ancient documents, and discovered that the soldier had, indeed, taken part in several campaigns in South America.

Torture

Don Aldro Valdez was involved in terrible bloodshed.

"Thousands of Indians were tortured and slain", said the man brought back to life from the dead with the extract from the tree of life.

"All we were concerned about was the pursuit of gold. We rampaged throughout the land, slaughtering and stealing and amassing a tremendous hoard of treasure.

"But life was hard. Many of my men died from disease and from surprise attacks by the Indians.

"Eventually, I suffered a mortal wound myself in a battle to overthrow a local chieftain.

"I clearly recall a spear being thrust through my chest, the sharp stab of pain. Then nothing. I had no recollection of these events until my recent death", the man recalled.

"Then I was treated with the extract from the tree of life, and they all came flooding back."

Wynn-Hughes heard from a woman how, in an earlier life, she'd lived in his native Wales.

Enlisting the aid of relatives, he discovered that a woman by the name he'd been given had lived in the mining village of Aberfain at one time.

"All the men in my family were colliers", the woman recalled. "Times were hard. Their pay terrible. But somehow we managed to survive.

Humble

"Clearly now, I can recollect the humble cottage where we lived, the grime-covered streets and the towering pit heads.

"It just seemed to hit me after I was given the tree extract."

The extract is obtained from a tree with the colloquial name of Black Man's Head.

It's long been recognized as a snake-venom antidote and has often been used by natives to resurrect animals that have suffered attacks from poisonous snakes.

It's only recently, however, that successful experiments have been carried out on humans, the first in Sao Paulo and Rio de Janiero, Brazil.

U.S. and Japanese chemists have been able to synthesize the plant's active ingredients, but they still don't know the identity of the natural source.

"At least a dozen plants share the name of Black Man's Head, or Cabeza de Negro, explains Koji Nakanishi of Columbia University.

"Unfortunately, the woman who supplied us with the extract wouldn't tell us which one it came from."

Nakanishi's team was able to isolate and describe the structure of the antidote molecule.

Tests

Then other scientists tested it on mice and dogs that had been given two to three times the lethal dose of venom from a snake known as the fer-de-lance.

The fer-de-lance is found throughout the tropics, and its bite usually is fatal to humans.

The research scientists found that the antidote successfully restores heartbeat, blood pressure and respiration to normal within approximately two hours.

Nakanishi believes that the antidote may be effective against venom from many other snake species as well.

Scientists were given a clue to its incredible properties when animals were seen to seek out the tree after they'd been bitten by a snake.

It's thought that just rubbing against the plant might reward some animals with total immunity against snake bites

Astounding human experiments show that not only is the extract extremely potent in its use against snake bites, it also has the fantastic power to halt and reverse a number of degenerative diseases.

It also enabled those who took part in the experiments to recall experiences they didn't even realize they had experienced.

"Incredible as it may sound, it's been found to act even after men and women have been pronounced clinically dead", says Wynn-Hughes.

"It's uncertain at this stage in time whether it'll work on every person who's died. But it's certain that the extract can restore life in a number of cases.

"It would appear that it's likely to prove most effective in bringing back to life children and adolescents who haven't been subjected to long-term diseases or who've died in accidents."

Now, scientists can synthesize the amazing compound. They'll soon start manufacturing batches in laboratories throughout the world.

But Dr Alfred Spiegel, an associate of Wynn-Hughes, cautions: "It may well be some years before the compound can be made generally available.

"In the United States, Britain, Japan and other Western nations, it'll hve to be subjected to extensive testing before it can be made available to doctors and hospitals.

"South American physicians have reported success in dozens of cases. And now attempts will be made to replicate their fantastic results in America and England.

"The discovery of the compound is probably the most exciting breakthrough in medicine since the discovery of penicillin."

PATENTS

US4429141
PHYSIOLOGICALLY ACTIVE PTEROCARPAN-COMPOUNDS,METHODS FOR THE ISOLATION THEREOF AND THERAPEUTICALLY ACTIVE COMPOSITIONS CONTAINING THEM

[PDF]

Inventor: DARKO LASZLO L // NAKANISHI KOJI (+1)
Applicant: RICHTER GEDEON VEGYESZET

Physiologically active crystalline products of manufacture having the formulas: (I) and (II) are described. These compounds are effective against the toxic venoms of poisonous snakes, spiders and other insects, and against E. coli endotoxins; methods are described for isolation of the above compounds.

BACKGROUND OF THE INVENTION

1. Field of the Invention

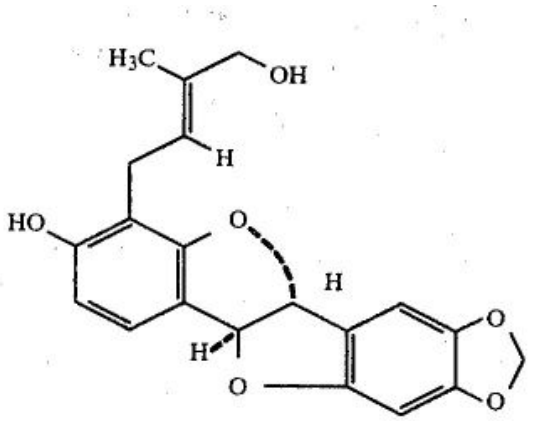
This invention relates to products of manufacture which are substantially pure crystalline pterocarpan compounds which have been isolated from the crude extracts of natural products and to methods for isolating the compounds.

2. Description of the Prior Art

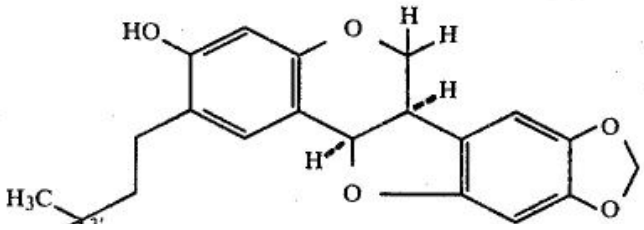
It has been known that the aqeuous alcoholic extract of the root of the South American cabeca de negra tree has been available to plantation workers in the upper Amazon jungle as an oral antidote against snake and spider venoms. About ten varieties of the species cabeca de negra are known in South America.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

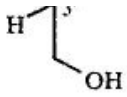
SUMMARY OF THE INVENTION

The invention includes the following products of manufacture which have been isolated in substantially pure crystalline form: ##STR2## For convenience, the compounds of the above structure will be referred to hereinafter as cabenegrin I and cabenegrin II, or as compounds (I) and (II), respectively. The invention also includes the step-wise method employed to recover and isolate the essentially pure crystalline products referred to as cabenegrin I and cabenegrin II.



and





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. That is, these compounds act as potent antidotes against effects of snake and insect toxins. The compounds (I) and (II) are also useful in treating the effects of other known organic toxins such as E. coli endotoxin and that produced by Clostridium botulinum, commonly referred to as botulism or food poisoning. The details of tests establishing the utility of compounds I and II are described in a co-pending application filed concurrently herewith in the name of Lazslo Darko under U.S. Ser. No. 358,191, and the entire disclosure of that application is incorporated herein by reference. 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 particularly 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 in liquid form as a suspension or solubilized in a compatible pharmaceutical carrier. The pure crystalline material can be administered orally in capsule or tablet form 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 is available to determine the effects of the therapy. If desired compounds (I) and (II) can be mixed together in various proportions, and the mixture prepared as described above with suitable pharmaceutical carriers.

DETAILED DESCRIPTION OF THE INVENTION

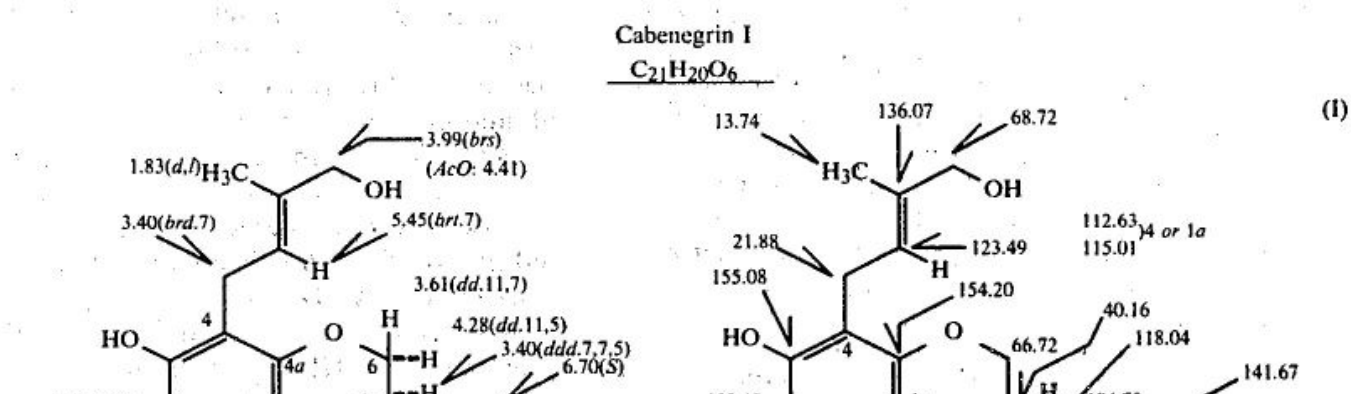
Approximately 1/2 kilo of the washed root of the cabeca de negra tree is chopped into small pieces which may then be mascerated, pulverized or otherwise treated to break down the fibrous structure. This step can be accomplished in a blender or laboratory homogenizer. Roots evidencing mold or fungus should not be used. The pulverized root is placed in a large glass beaker or other suitable vessel which may be covered and subsequently stirred. A sufficient quantity of ethanol:water 77:23 is added to cover the pulverized root and briskly stirred for 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 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.

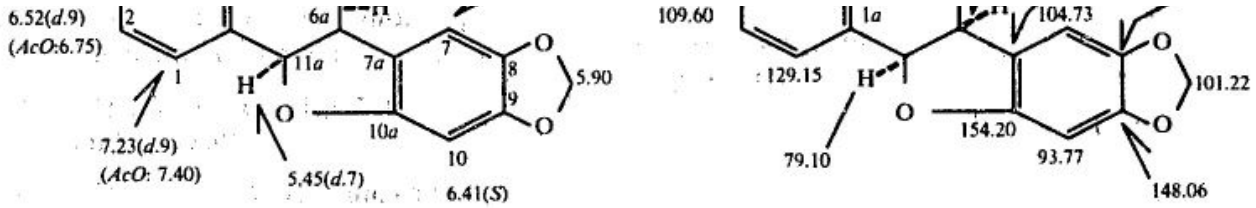
The following procedure was employed to isolate the active compounds from the crude extract as prepared above.

The aqueous ethanol crude extract (135 ml) was concentrated by gentle 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 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 were 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, and the water layer was discarded.

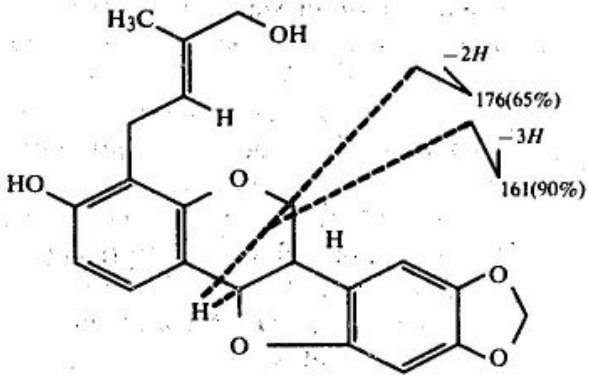
The ether layer extracted above is subjected to high pressure liquid chromatography (HPLC) on Sephadex LH-20 as a first step and as the next step on silica gel using aqueous methanol as the eluting solvent. This procedure results in two fractions, and the first is further separated by HPLC employing 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 DEG-168 DEG C. is obtained and analysis shows the composition to be C21 H20 O6. The Rf value of compound I on thin layer chromatography employing silica gel CO/Kiesel guhr F-254 and benzene/ethyl acetate/methanol (15/4/1) was 0.53.





E1(18eV)
368(M^+, 50%)
350(M^+-H_2O, 100%)
335(M^+-H_2O-Me, 95%)



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

UV (in MeOH): 209 nm (E 75,000), 233 nm (shoulder, E 24,000), 309 nm (E 13,000)

CD (in MeOH): 213 nm (E -25.58), 220 nm (.DELTA.E -2.00), 238 nm (.DELTA.E -9.84); 302 (.DELTA.E +3.15)

IR (in CHCl3): 3550 cm@-1, 1600 cm@-1, 1113 cm@-1, 925 cm@-1.

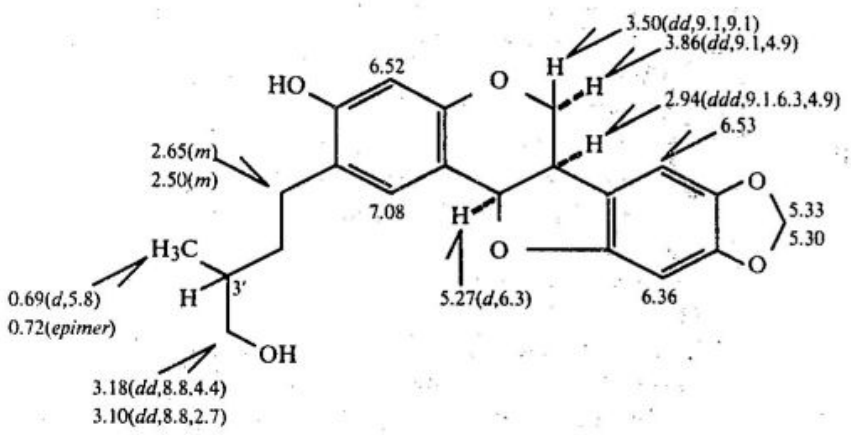
In addition to these spectral data, the following @1 H-NMR, @13 C-NMR, and MS (E1) data were measured; these spectroscopic measurements led to the depicted structure.

PMR, CMR and MS of Compound (I): ##STR3##

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 .mu.-Bondapak C18 and methanol/acetonitrile/H2 O/n-PrOH (71/71/59/2). Compound (II) was obtained in essentially pure crystalline form in a yield of about 1 mg. The structure of this compound is based on the following physical constants: ##STR4##

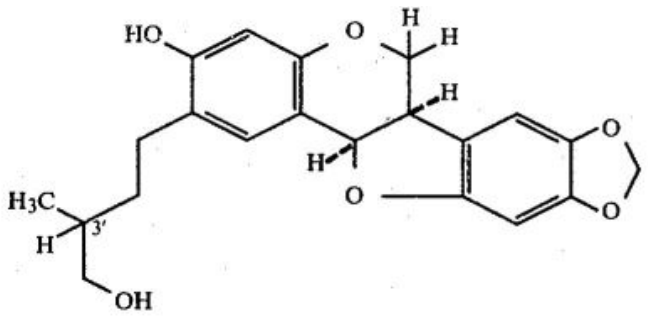
Cabenegrin II
$C_{21}H_{22}O_6$ (E1-MS)

(II)



UV(MeOH): 204 nm(ϵ116,000) CD(MeOH): 237 nm(Δϵ−6.68)
230 nm(ϵ8,000) 280 nm(Δϵ−0.46)

292 nm(ε9,400) 299 nm(Δε+1.72)
308 nm(ε11,800)



(II)

The structure of compound (II) is shown by NMR data to be a 3:1 mixture of epimers at C-3'. The structures of both compounds cabenegrin I and cabenegrin II have been confirmed by synthesis of their respective racemates.

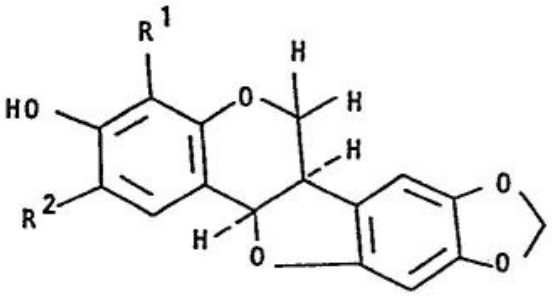
EP0089229

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

[PDF]

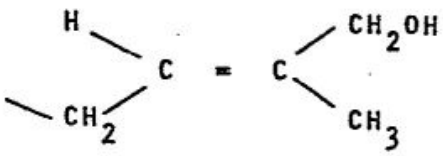
The invention relates to physiologically active pterocarpan-compounds and methods for the isolation thereof. These compounds have the formula I <CHEM> wherein R<1> represents a hydrogen atom and R<2> represents a -CH2CH2-CH(CH3)-CH2OH group, or R<1> represents a <CHEM> and R<2> represents a hydrogen atom, together with all optical isomers and racemic mixtures thereof. These compounds 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.

These compounds have the formula I



(I)

wherein R^1 represents a hydrogen atom and R^2 represents a $-CH_2CH_2-CH(CH_3)-CH_2OH$ group, or R^1 represents a



group

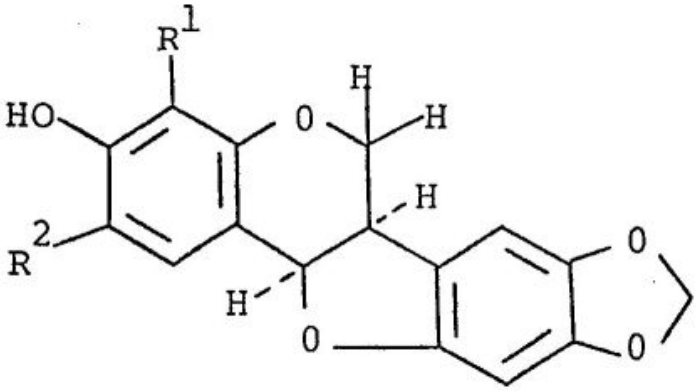
and R^2 represents a hydrogen atom, together with all optical isomers and racemic mixtures thereof. These compounds

[0001] 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 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.

[0002] It is known that the aqueous alcoholic extract of the root of the South American cabeca 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 cabeca de negra are known in South America. Neither the nature of the active components nor

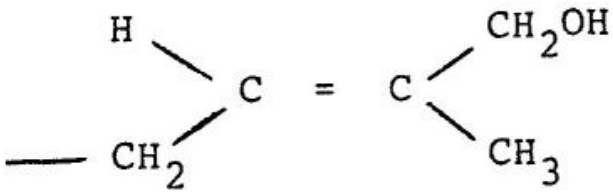
studies of the side effects, pharmacological activity, stability and the like are known to have been published on the compounds of the invention. Pterocarpans possessing antimicrobial properties have been identified in the literature, see Heterocycles, Vol. 15, 1163 [1981].

[0003] In one aspect, the invention relates to pterocarpan compounds having the formula (I)



(I)

wherein R<1> represents a hydrogen atom and R<2> represents a -CH2CH2-CH(CH3)-CH20H group, or R represents a



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

[0004] 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-hydroxymethyl-butene-2)-8,9-methylenedioxy-pterocarpan] 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 I and cabenegrin II.

[0005] 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 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 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 where paralysis of the respiratory system is manifested. Treatment against the effects of cardio-vascular toxins is also indicated.

[0006] 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 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 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.

[0007] A crude extract from which the compounds of the invention may be obtained may be prepared as follows:

[0008] Approximately 2 kilo of the washed root of the cabeca de negra tree is chopped into small pieces 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 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 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 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.

[0009] The following procedure may then be employed to isolate the active compounds from the crude extract as prepared above.

[0010] 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.

[0011] 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.

[0012] 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[deg.]C is obtained and analysis shows the composition to be C21H20O6. 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.

[0013] The U.V., C.D., and I.R. of compound I are as follows:

[0014] UV (in MeOH): 209 nm (e 75,000), 233 nm shoulder, ([epsilon] 24,000), 309 nm (e 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 CHC13): 3550 cm<-1>, 1600 cm<-1>, 1113 cm<-1>, 925 cm<-1>.

[0015] In addition to these spectral data, the following <1>H-NMR, <13>C-NMR, and MS (El) data were measured concerning cabenegrin I:
<1>H-NMR: 1.83 (3H, d, 1 Hz, 4'-CH3);
3.40 (2H, br, d, 7 Hz, C l');
3.40 (2H, ddd, 7, 7.5 Hz, C 6a);
3.61 (2H, d, d, 11.7 Hz, c 6[beta]);
3.90 (2H, -OCH20);
3.99 (2H, br, s, -CH2-OH);
4.28 (lH, dd, 11.5 Hz, C 6a);
5.45 (lH, br, t, 7 Hz, C 2');
5.45 (lH, d, 7 Hz, C lla);
6.41 (1H, s, C 10);
6.52 (lH, d, 9 Hz, C 2);
6.70 (1H, s, C 7);
7.23 (lH, d, 9 Hz, C 1) ppm.
<13>C-NMR: 13.74 (4'-CH3) ; 21.88 (C 1'); 40.16 (C 6a);
66.72 (C 6); 68.72 (-CH2-OH); 79.10 (C lla);
93.77 (C 10); 104.73 (C 7); 109.6 (C 2);
112.63 (C 4 or C la); 115.01 (C 4 or C la);
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);
155.08 (C 3) ppm.
MS (18 eV):
M<+ >= 368, 50%
m/e = 350, 100%; 335, 95%; 335, 95%; 176, 65%; 161, 90%.

[0016] The second sample of material recovered from the HPLC described above comprised an oily mixture of approximately 10 mg. This second active fration, 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 /u-Bondapack C18 and methanol/ 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'

[0017] <1>H-NMR: 0.69 (3H, d, 5.8 Hz, 3'-CH3);
2.50 (2H, m, C 1');
2.67 (2H, m, C 1');
2.94 (lH, ddd, 9.1, 6.3, 4.9Hz, C 6a);
3.10 (2H, dd, 8.8, 2.7 Hz, -CH2-OH);

3.18 (2H, dd, 8.8, 4.4 Hz, -CH2-OH);
3.50 (lH, dd, 9.1, 9.1 Hz, C 6[beta]);
3.86 (lH, dd, 9.1, 4.9 Hz, C 6a);
5.27 (lH, d, 6.3 Hz, C lla);
5.30 (2H, -OCH20-);
5.33 (2H, -OCH2O-);
6.36 (lH, C 10);
6.52 (lH, C 4);
6.53 (lH, C 7);
7.08 (lH, C 1) ppm.

[0018] UV (in MeOH):204 nm ([epsilon] 116,000), 230 nm ([epsilon] 8,000), 292 nm ([epsilon] 9,400), 308 nm ([epsilon] 11,800).

[0019] CD (in MeOH): 237 nm ([Delta][epsilon] -6.68), 280 nm ([Delta][epsilon] -0.46), 299 nm ([Delta][epsilon] -1.72) .

[0020] The structure of this compound is shown by NMR data to be a 3:1 mixture of epimers at C-3' and 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.

[0021] 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.

[0022] 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.

[0023] 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.

[0024] 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.

[0025] 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 I and II to normal healthy (i.e. non-envenomated) animals.

[0026] Administration does not significantly alter resting action potentials, end plate potentials, nerve impulse transmission, neuro-muscular function or brain wave activity in experimental animals.

1. Antidotal effects against snake venom in dogs.

[0027] Envenomation by-a lethal dose of snake venom, such as Bothrops atrox, Crotalus adamamteuns, or Crotalus atrox, produces a precipitous fall in arterial blood pressure, a decrease of heart rate and en 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 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 alpha and the beta rhythm of the brain (EEG). This change is also associated with a decrease in impulse transmission over the motor verves and progressive blockage of the neuromuscular apparatus which is similar to that produced by curare. Venoms had no effect on muscle response to direct stimulation.

[0028] 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 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.

[0029] Within 15 minutes following envenomation, marked decreases in heart rate and blood pressure are consistently noted. At from 15 to 30 minutes, respiration likewise descreases from an average of 20 per minute to 5 per minute. Treatment is initiated when severe cardio- vascular embarrassment and apparent respiratory difficulties are observed, usually at from 15-30 minutes following envenomations.

[0030] 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 ethanol (15:75) is prepared. Doses are prepared for administration by stirring 5 ml of each of the respectve alcoholic solutions of the compounds of the invention into 50 ml of water.

[0031] Administration of the respective solutions 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 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 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 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

[0032] Three adult beagle dogs are used to demonstrate the effectiveness of the cabenegrin I and II in treating 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 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 collapse and respiratory difficulties appear. These usually occur within about 112 hours after the injection of the toxin.

[0033] Solutions of cabenegrin I and II, prepared as described above, are administered by stomach tube 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

[0034] 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 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 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%.

[0035] When either of the compounds is given following. 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

[0036] 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.

[0037] Snake venom decreases both brain wave activity 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 of the compounds. This is followed by a slow, gradual return to control levels after from 12 to 24 hours.

[0038] In certain experiments in which complete neuro- muscular blockage occurs and the animals are no longer capable of spontaneous respiration, artificial ventilation 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.

[0039] Cortical electrical activity is markedly (25-30%) 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.

[0040] Results of these studies indicate that oral 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.

[0041] 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.

[0042] 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 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.

[0043] Novel compositions for oral administration 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 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.

[0044] In many instances, either the nature of the poisonous toxin, or the type of deteriorating condition 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 deterioration will require administration of oral doses via stomach tube or intravenous injection by syringe or catheter.

[0045] A pharmaceutical composition was prepared by blending the following materials in the specified proportions by weight:

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.

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Method of treating mammals for effects of neuro- and cardiovascular toxins

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Abstract

Methods of treating mammals, including man, for poisonous snake and insect bites, E. coli endotoxins, botulism and other neurotoxins and cardiovascular toxins by administering therapeutic quantities of physiologically active compounds of the formula: (I) and (II) and the materials from which they are derived.

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

This invention relates to antidotes for treating the effects of poisonous snake and insect bites in mammals, including man. It also relates to the use of products of manufacture in 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 with the products of manufacture and with aqueous alcoholic extracts of the natural products from which they are derived.

SUMMARY OF THE INVENTION

The invention includes therapeutically active compositions for the treatment of mammals comprising compounds selected from the class of: ##STR2## and pharmaceutical carriers. The invention also includes methods of treating mammals for the effects of neuro toxins and cardiovascular toxins which comprise administering to a mammal a therapeutic quantity of a composition consisting of compounds selected from the class of compounds (I) and (II) set forth above and pharmaceutical carriers. The invention disclosed herein also includes the treatment of specific symptoms in mammals, including humans, with the aqueous alcoholic extracts, or their equivalents, of the natural materials from which they are derived.

The methods for producing cabenegrin I and cabenegrin II in substantially pure crystalline form are described in a co-pending application filed concurrently herewith in the names of Laszlo Darko, Koji Nakanishi and Masachi Nakagawa, under U.S. Ser. No. 357,805, entitled Physiologically Active Compounds and Their Isolation, and the entire disclosure of that application is incorporated herein by reference. That application describes in detail an extraction scheme whereby, as new products of manufacture, crystalline compounds corresponding to compounds (I) and (II) and referred to as

cabenegrin I and cabenegrin II, respectively, are obtained, that scheme may be summarized as follows:

About one-half kilo of the cleaned root from the cabeca de negra tree is chopped and further comminuted to break down the fibrous structure. The pulverized root is placed in a container with a sufficient quantity of ethanol:water (77:33) to cover it, and allow to stand, with occasional stirring for about two days. The aqueous alcoholic solution is separated from the root and reduced by gentle warming under vacuum by to brown oily residue. This concentrate is treated with 50% aqueous methanol and the solution extracted with hexane. The hexane layer is discarded and the water layer is extracted with ether. After separation the ether layer is subjected to high pressure liquid chromatography (HPLC) on a column of Sephadex LH-20 and silica gel, using aqueous methanol as the eluting solvent. Two fractions are obtained. The first fraction is subjected to further HPLC and compounds (I), also referred to as cabenegrin I, is obtained as a substantially pure white crystalline material having a sharp melting point at 167 DEG-168 DEG C. The second fraction described above is an oily material which is subjected to further HPLC and compound (II), also referred to as cabenegrin II, is obtained as a crystalline material.

In the above extraction scheme, 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 20-25 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 or compounds (I) and (II) 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 fraction. 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 intra muscular 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 I and II to normal, healthy (i.e., non-envenomated) animals.

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

The following tests demonstrate the treatment with cabenegrin I and II of the effects of toxins which affect either the cardiovascular and/or the neurophysiological systems of experimental animals.

ANTIDOTAL EFFECTS AGAINST SNAKE VENOM IN DOGS

Envenomation by a lethal dose of snake venom, such as Bothrops atrox, Crotalus adamamteuns, or Crotalus atrox, produces a precipitous fall in arterial 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 or peripheral 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 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 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 anesthetized with Na pentobarbitol (30 mg/kg) and monitored for changes 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 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 following envenomation).

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 ethanol (15:75) is prepared. Doses are prepared for administration by stirring 5 ml of each of the respective alcoholic solution of compounds (I) and (II) into 50 ml of water.

Administration of the respective solutions 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 of the respective alcoholic solution of compounds (I) and (II), 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 vital signs return to within 10% of control.* 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 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.

*The effective dose range is between 10 to 20 ml of antivenom extract per animal.

ACTIVITY OF CABENEGRIN II AND II AGAINST E. COLI ENDOTOXIN

Three adult beagle dogs are used to demonstrate the effectiveness of the cabenegrin I and II in treating shock caused by E. coli endotoxin. The dogs are anesthetized with Na pentabarbital (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 IV into a catheter placed in the vein of the hind limb of the dogs. In the first experiment no antidotal therapy was initiated and the animal expired at 2 hours after injection. In the three additional cases antidotal therapy is initiated at the time when severe cardiovascular collapse and respiratory difficulties appear. These usually occur within about 11/2 hours after the injection of the toxin.

Solutions of cabenegrin I and II, prepared as described above, are administered by stomach tube 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.

ISOLATED HEART (LANGENDORFF) PREPARATION

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 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 following the administration of venom. When a solution containing 0.5 mg/ml of either compound (I) or compound (II) 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 percent.

When either compound (I) or compound (II) is given following 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.

NEUROPHYSIOLOGICAL FUNCTION

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 and nerve impulse transmission. These are restored to near, 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 of the compounds. This is followed by a slow, gradual return to control levels at 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 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 (25-35%) 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 or IV 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.

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

Novel compositions for oral administration 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 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 or deteriorated condition 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 signs are in an advanced stage of 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 proportions by weight:
Compound I: 20
Starch: 15.0
Magnesium stearate: 2.0
Sodium benzoate: 6.0
Benzalkonium chloride: 2.0

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