PERGAMON

Phytochemistry 55 (2000) 627–642

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Plant natural products active against snake bite — the molecular approach

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Received 7 January 2000; received in revised form 8 May 2000

Abstract

The article surveys the substances identified in plants reputed to neutralize the effects of snake venoms. Protective activity of many of them against the lethal action of the venom of the jararaca (*Bothrops jararaca*) snake was confirmed by biological assays. It was shown that all belong to chemical classes capable of interacting with macromolecular targets — receptors and enzymes. In a few cases it has been shown that exogenous natural micromolecules can mimic the biological activity of endogenous macromolecules. From the evidence presented, it can be inferred that micromolecules which neutralize the action of snake venoms mechanistically replace endogenous antitoxic serum proteins with venom neutralizing capacity such as produced by some animals.

Keywords: Natural products against snake venom; Snake venom antidotes

1. Introduction

The use of plants against the effects of snake bite has long been recognized, (Bocquillon-Limousin, 1891) even in modern times but only for the last 20 years has it merited closer scientific attention (Nakagawa et al., 1982; Rizzini et al., 1988; Siddiqui and Husain, 1990; Selvanayagam et al., 1995; Reyes-Chilpa and Jimenez Estrada, 1995; Pereira et al., 1996). Several reviews about the subject have by now been published (Mors, l991; Martz, 1992; Houghton and Osibogun, 1993; Selvanayagam et al., 1994, 1995; Taube, 1994). But, while quite a number of reports, from different geographical areas, mention plants reputed to neutralize the action of snake venom, only a few attribute such activity to certain chemical compounds identified in them (Pereira et al., 1994; Schwarz, 1995), and even less are concerned with a possible mechanism of action (Gowda, 1997).

In a previous publication (Pereira et al., 1994), we reported our results in testing a number of substances isolated from such plants against the lethal action of the venom of jararaca (*Bothrops jararaca*) snakes, on mice. Our procedure differs from most others in that the substances were administered by the oral route, prior to envenomation, thus simulating the popular use by which plants or their extracts are ingested, even prophylactically.

These experiments have been continued and we herewith wish to present the results and the conclusions which can be drawn from them, and from data recollected from the literature. Specific pharmacological activities, such as antihemorrhagic, antineurotoxic, anticoagulant, antimyotoxic, anticardiotoxic, and others, will not be considered in the present paper. The only parameter here is the anti-lethal effect. Such a dissociation may, at first sight, seem an oversimplification. But it is justified, not only by the widespread popular use of the plants, which has in view solely the saving of lives in the field, but it also makes sense when one considers that in this practice the people make no distinction between the nature of the offending snakes and, therefore, between the multitude of possible toxic components of the venoms. For completeness it should be mentioned that in the majority of cases the mashed parts of the reputed plants

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0031-9422/00/$ - see front matter
PII: S0031-9422(00)00229-6

are also placed on the area of the bite, in the form of cataplasms.

In the following paragraphs, the compounds will again be ordered under chemical categories. The relative activities of all these vary; but considerably active ones can be found in all of them. One now comes back to the question which has been put forward before: what do all these compounds have in common? It is the purpose of this article to show that they all belong to classes of 'secondary metabolites' capable of interacting with macromolecular targets — receptors and enzymes.

2. Materials and methods

Experimental animals were albino mice of 20 g average weight. As before, the venom was supplied by Instituto Vital Brazil, Niterói, RJ. DL_{100} was equal to 20 mg per kg body weight, different, therefore, from the batch used in our earlier experiments ($DL_{100} = 5$ mg). The adopted procedure was the same as described in the previous publication: the substances tested, in aqueous solution or suspension, if necessary with the aid of an emulsifier (Tween), were administered by gastric intubation, at the dose of 100 mg per kg body weight, 1 h prior to envenomation by subcutaneous injection of the venom — the most appropriate time interval in accord with our experiments.

3. Results and discussion

The results of the observed protection are given in percentage values representing the proportion of surviving animals in each test group. Readings were annotated 6, 24 and 48 h after envenomation, but, in the present paper, only the percentage of the final survivors, after 48 h, is indicated in parentheses after the name of each substance. The surviving animals showed only slight hemorrhage at the site of injection of the venom, but otherwise showed normal behaviour. The present paper includes results already reported in the previous publication, although the inevitable variation of activity of the snake venom from batch to batch does not allow a rigorous comparison between data. Even if 100% protection has not been achieved with isolated compounds, but only with plant extracts, the fact that there are survivors at all is significant, since in the corresponding control groups all the animals died. It should also be kept in mind that one single constituent does not reproduce the full activity of an extract. As is the case with many medicinal herbs, there is, admittedly, more than one active compound present in the plants, acting synergistically on several target structures.

An interesting observation in this respect has been reported by Honda et al. (l986), in studying the sedative effect of the leaves of *Perilla frutescens* (which are also known as an antidote against snake venom). The authors found that the plant contains, besides perillaldehyde, the steroids sitosterol, stigmasterol and campesterol. It was concluded that the sedative activity of the leaf extract is caused by the combined effects of perillaldehyde and stigmasterol. Other combinations of the components did not show the same result.

The fact that the identified active substances are mostly low molecular weight compounds showing some kind of biodynamic activity has already been pointed out at the beginning of our studies (Mors, 1991). Many of them are considered 'multifunctional', in the sense that more than one biochemical or pharmacological property has been demonstrated for them, usually independently by different authors. A striking parallelism exists between the capability of plants and their chemical components of neutralizing the actions of snake venoms, and anti-inflammatory and anti-hepatotoxic properties. This observation suggests the existence of some analogy between the mechanism which governs these activities. In many cases, the observed multifunctionality has found an explanation in the capacity of such compounds to bind to proteins, and thus interfere with the natural functions of many biologically active macromolecules.

The multifunctional biodynamic activity of many apparently trivial, ubiquitous micromolecular natural substances — especially non-nitrogen containing ones — may seem surprising at first sight. But in reality it is not. Several monoterpenes, cinnamic acid and benzoic acid derivatives, in the molecular weight range between 150 and 200, are of ecological significance. Released into the soil by many plants, they inhibit the growth and development of others. Some are feeding deterrents produced by plants and insects. Ageratochromene (molecular weight just below 200) is a hemiterpene affecting the molting behaviour of insects. In higher animals, the effect of camphor on the central nervous system can be recalled. Anesthetic action of phenylpropanoids, like eugenol derivatives, has been demonstrated. All these, and many more examples, prove that such compounds are able to strongly interfere with the chemistry of living beings.

The time has come to apply this knowledge to the field of anti-snake venoms. Without doubt, the polypeptidic nature of their toxins and enzymes offers a multitude of targets. In a few instances, like in α-bungarotoxin, active sites have actually been identified. In a number of cases the interaction of the active substances with certain enzymes has been demonstrated in vitro, with interference in enzyme-substrate complex formation. Still other palpable points of attack are metal atoms which are integral parts of enzyme receptors. Evidently, much work remains to be done in order to untangle the many possibilities of functional inhibition

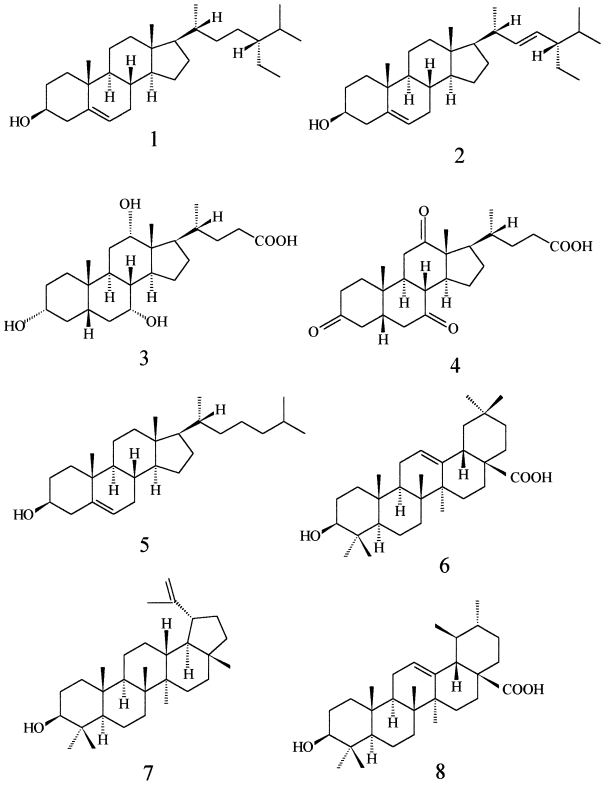
in this field. It is the purpose of this paper to show how the great diversity of chemical structures capable of neutralizing snake venom supports the concept.

Complete botanical names, including the authorities for the binomials and the respective families, parts of plants used and citations referring to the anti-snake venom activity, are given in Table 1.

4. Steroids and triterpenes

4.1. Model compound 1: sitosterol (*1*, 70% protection)

Sitosterol (β-sitosterol) is the most abundant of the phyto-steroids. The compound occurs either as such or in the form of its glucoside ('sitosterolin'), frequently accompanied by its mono- unsaturated analogue, stigmasterol **2**, also either free or as its glucoside. Administered to animals and humans, as well as in in vitro tests, sitosterol has been found to display a large array of pharmacological properties, among them anti-inflammatory action, as mentioned above. As a medicinal agent it has been used for lowering plasma cholesterol (now abandoned) and for checking benign prostatic hyperplasia (still in use). In the following are enumerated just a few of the scores of plants reputed for anti-snake venom activity in which sitosterol or its glucoside were found to be present, usually accompanied by its analogue, stigmasterol, in varying proportions (not counting other constituents).



Achillea millefolium; *Aegle marmelos*; *Aristolochia serpentaria* (the Virginia snakeroot); *Caesalpinia bonduc*; *Calendula officinalis*; *Cissampelos glaberrima* (the popular name in Brazil, 'cipó-de-cobra', means snake liana); *Cocculus hirsutus*; *Cynanchum paniculatum* (considered the main anti-snake bite plant in China); *Eclipta prostrata* (a traditional drug against snake bite both in China and in Brazil); *Euphorbia hirta*; *Gloriosa superba*; *Marsypianthes chamaedrys* (the popular name 'boia-caá', of tupi origin, meaning snake plant); *Ocimum basilicum*; *Ophiorrhiza mungos* (the Latin name of the genus, given by Linnaeus, means snake root); *Oldenlandia diffusa*; *Pluchea indica*; *Pothomorphe umbellata*; *Prestonia coalita*; *Serenoa repens*; *Sophora subprostrata*; *Taraxacum officinale*.

The capacity of steroids for complex formation has been recognized in many cases. Paradigms are the bile acids and their salts (e.g. cholic acid **3**). These substances form stable molecular addition compounds with many organic molecules, including aliphatic hydrocarbons, alcohols, ethers, carboxylic acids and aromatics. These associations, known as choleic acids, contain the two components in a fixed molecular ratio. The physiological importance of these steroids lies in their capacity to convert fats and fatty acids into water soluble or emulsifiable compounds and thus facilitate their intestinal absorption, their hydrolysis and the absorption of the fat soluble vitamins.

The mentioned complexes are held together by van der Waals and hydrophobic forces and in this consists the analogy which can be proposed for the behaviour of sitosterol. Although in this case investigations are still lacking, here, also, molecules of an extended shape carrying a hydrophilic group at one end, can associate with hydrophobic ones, surrounding them and, with the hydrophilic groups turned to the outside, form molecular complexes with their own physico-chemical properties.

4.2. Dehydrocholic acid (*4*, 80% protection)

Dehydrocholic acid (3,7,12-trioxocholanic acid), although not a naturally occurring compound, was included in this study on account of its belonging to the series of the bile acids. Still much used as a choleretic, the substance is obtained industrially by oxidation of cholic acid. The high degree of protection which it confers against the action of jararaca venom is in accord with the above reasoning. Bile acids, however, found in the liver, bile and intestine of animals, are not encountered in plants.

4.3. Cholesterol (*5*, 60% protection)

Cholesterol is not a typical constituent of plants; but it does occur in plants occasionally and has even been

Table 1
Plants reputed active against snake bite venom and the respective sources of ethnological information

Acacia catechu Willd.	Mimosaceae	Bark	Siddiqui and Husain, 1990
Acacia farnesiana Willd.	Mimosaceae	Root	Jaccoud, 1954
Acacia leucophloea Willd.	Mimosaceae	Bark	Selvanayagam et al., 1995
Acacia polyacantha Willd.	Mimosaceae	Root	Hedberg et al., 1983
Achillea millefolium L.	Asteraceae	Whole plant	Duke and Ayensu, 1985
Achyranthes aspera L.	Amaranthaceae	Whole plant	Jain and Puri, 1984
Aegle marmelos Correa	Rutaceae	Root	Pitre and Srivastava, 1987
Agrimonia eupatoria L.	Rosaceae	Root	Duke, 1985
Ajuga decumbens Thunb.	Lamiaceae	Seeds	A Barefoot..., 1977
Allium cepa L.	Liliaceae	Skin of bulb	Morton, 1981
Alstonia boonei Willd.	Apocynaceae	Bark, leaves	Datta and Datta, 1984
Arctium lappa L.	Asteraceae	Seeds, root	Duke, 1985
Argemone mexicana L.	Papaveraceae	Root	Silva, 1979
Aristolochia serpentaria L.	Aristolochiaceae	Root	Duke, 1985
Belamcanda chinensis DC	Iridaceae	Root	A Barefoot..., 1977
Berkheya spekeana Oliv.	Asteraceae	Root	Agoro, 1978
Boehmeria nivea Gaud.	Urticaceae	Root	Duke and Ayensu, 1985
Bredemeyera floribunda Willd.	Polygalaceae	Root	Wasicky and Ferreira, 1949
Brongniartia podalyrioides H.B.K.	Fabaceae	Root	Reyes Chilpa et al., 1994
Brunfelsia grandiflora *D. Don*	Solanaceae	Root, bark	Lloyd et al., 1985
Brunfelsia uniflora D. Don	Solanaceae	Root	Yer et al., 1977
Buddleja brasiliensis Jacq.	Buddlejaceae	root	Silva, 1979
Byrsonima crassifolia H.B.K.	Malpighiaceae	Whole plant	Morton, 1981
Caesalpinia bonduc Roxb.	Caesalpiniaceae	seeds	Silva, 1979
Calendula officinalis L.	Asteraceae	flowers	European folk wisdom
Casearia sylvestris Sw.	Flacourtiaceae	root	Barbi, 1992
Cassia tora L.	Caesalpiniaceae	root	Sahu, 1984
Centipeda minima Br. et Asch.	Asteraceae	Whole plant	A Barefoot..., 1977
Chenopodium ambrosioides L.	Chenopodiaceae	Whole plant	Jain and Puri, 1984
Chiococca alba Hitch.	Rubiaceae	Root	Morton, 1981
Cimicifuga racemosa Nutt.	Ranunculaceae	Root	Hussey, 1974
Cissampelos glaberrima St.Hil.	Menispermaceae	Root	Pio Corrêa, 1926–1975
Cocculus hirsutus Diels	Menispermaceae	Root, bark	Pushpangadan and Atal, 1984
Coffea arabica L.	Rubiaceae	Root	Morton, 1981
Combretum leprosum Mart. & Eichl.	Combretaceae	Bark	Facundo et al., 1993
Crataeva benthami Eichl.	Capparaceae	Whole plant	Amazonian lore
Cryptolepis sinensis Merr.	Asclepiadaceae	Root	Xiao Peigen, 1981
Curcuma longa L.	Zingiberaceae	Rhizome	Bisset and Mazars, 1984
Cynanchum paniculatum Kitag.	Asclepidaceae	Whole plant	Duke and Ayensu, 1985
Daphne mezereum L.	Thymeliaceae	Root	Grieve, 1931
Daphne odora Thunb.	Thymeliaceae	Root	Baba et al., 1985
Diospyros kaki L.f.	Ebenaceae	Unripe fruit	Okonogi et al., 1979
Dipteryx odorata Willd.	Fabaceae	Seeds	Grenand et al., 1987
Dipteryx punctata Amshoff	Fabaceae	seeds	Grenand et al., 1987
Dorstenia brasiliensis Lam.	Moraceae	Root	Silva, 1979
Echinacea angustifolia DC	Asteraceae	Root	Beringer, 1911
Echinops amplexicaulis Oliv.	Asteraceae	Root	Agoro, 1978
Eclipta prostrata L.	Asteraceae	Whole plant	A Barefoot..., 1977
Ehretia buxifolia Roxb.	Ehretiaceae	Root	Selvanayagan et al., 1995
Elephantopus scaber L.	Asteraceae	Whole plant	A Barefoot..., 1977
Eryngium yuccifolium Michx.	Apiaceae	Root	Mathias, 1994
Eupatorium triplinerve *Vahl*	Asteraceae	Leaves	Morton, 1981
Euphorbia hirta L.	Euphorbiaceae	Whole plant	Grenand et al., 1987
Fagopyrum cymosum Meissn.	Polygonaceae	Root	A Barefoot..., 1977
Feronia limonia Swingle	Rutaceae	Root	Agrawal et al., 1989
Foeniculum vulgare Mill.	Apiaceae	seed	Duke, 1985
Gentiana lutea L.	Gentianaceae	Rhizome	Grieve, 1931
Gloriosa superba L.	Liliaceae	Bulb	Duke, 1985
Gymnema sylvestre R.Br.	Asclepiadaceae	Leaves	Selvanayanam et al., 1995
Harpalyce brasiliana Benth.	Fabaceae	Roots	Silva, 1979
Helianthus annuus L.	Asteraceae	seeds	Hussey, 1974
Hemidesmus indícus R.Br	Asclepiadaceae	Root	Alam et al., 1994

(continued on next page)

Table 1 (*continued*)

Heterothalamus psidioídes Less.	Asteraceae	whole plant	Silva and Frizzo, 1988
Impatiens balsamina L.	Balsaminaceae	Flower	Duke and Ayensu, 1985
Impatiens capensis Boj.	Balsaminaceae	Whole plant	Abebe, 1986
Ipomoea batatas Poir.	Convulvolaceae	Leaves	Morton, 1981
Liatris squarrosa Willd.	Asteraceae	Root	Grieve, 1931
Luffa operculata Cogn.	Cucurbitaceae	Fruit	Morton, 1981
Macfadyena unguis-cati Gent.	Bignoniaceae	Leaves	Morton, 1981
Marsdenia cundurango Reich.f.	Asclepiadaceae	Bark	Bocquillon-Limousin, 1891
Marsypianthes chamaedrys Ktze.	Lamiaceae	Whole plant	Freire Allemao, 1863
Merremia tridentata Hall.	Convolvulaceae	Leaves	Watt and Breyer, 1962
Mikania cordifolia Willd.	Asteraceae	Leaves	Morton, 1981
Mikania glomerata Spreng.	Asteraceae	Leaves	Silva, 1979
Morus alba L.	Moreaceae	Stem	Duke and Ayensu, 1985
Myrica rubra Sieb. Et Zucc.	Myricaceae	Stem bark	Sakurai et al., 1987
Nerium oleander L.	Apocynaceae	Seeds	Duke, 1985
Nicotiana tabacum L.	Solanaceae	Leaves, flowers	Duke and Ayensu, 1985
Ocimum basilicum L.	Laminaceae	Whole plant	Duke and Ayensu, 1985
Oldenlandia diffusa Roxb.	Rubiaceae	Whole plant	A barefoot..., 1977
Ophiorrhiza mungos L.	Rubiaceae	Root	Agrawal and Dhar, 1959
Pentaclethra macroloba Ktze.	Mimosaceae	Bark	Morton, 1981
Perilla frutencens Britt.	Laminaceae	Leaves	Honda et al., 1986
Phyllanthus amarus Scum.	Euphorbiaceae	Whole plant	Unander et al., 1991
Pinellia ternata (Thunb.) Breitenbach	Araceae	Rhizome	Suzuki, 1969
Pinus sylvestris L.	Pinaceae	Resin	Sezik et al., 1997
Plantago major L.	Plantaginaceae	Whole plant	Hussey, 1974
Polygala senega L.	Polygalaceae	Root	Tyler, 1987
Polygonum bistorta L.	Polygonaceae	Stem and root	Grieve, 1931
Pothomorphe umbellata Miq.	Piperaceae	Whole plant	Pio Corrêa, 1926
Prestonia coalita Woods.	Apocynaceae	Vine	South Brazil lore
Punica granatum L.	Punicaceae	Whole plant	Jain and Puri, 1984
Ruta graveolens L.	Rutaceae	Whole plant	Grieve, 1931
Rubus sp.	Rosaceae	Fruit	Hussey, 1974
Serenoa repens Small	Arecaceae	Fruit	Duke, 1985
Sophora subprostrata Chum	Fabaceae	Root	Duke and Ayensu, 1985
Strychnos colubrina L.	Loganiaceae	Wood	European lore
Strychnos decussata Gilg	Loganiaceae	Root	Arnold, Gulumian, 1984
Strychnos nux-vomica L.	Loganiaceae	Seeds	Duke, 1985
Strychnos spinosa Lam.	Loganiaceae	Fruit	Hedberg et al., 1983
Taraxacum officinale Weber	Asteraceae	Whole plant	Duke, 1985
Thymus vulgaris L.	Lamiaceae	Whole plant	Duke, 1985
Torresea cearensis Fr. Allem.	Fabaceae	Bark	Jaccoud, 1954
Verbascum thapsus L.	Scophulariaceae	Whole plant	Jain and Puri, 1984

identified in snake venom antidotes, like onion skins and the root of *Ehretia buxifolia Roxb*.

The capacity of cholesterol for complex formation became evident in the early nineteen hundreds, when R. Ransom observed that the addition of cholesterol destroys the violent hemolytic activity of the saponin digitonin. Shortly afterwards, A. Windaus found that cholesterol combines with digitonin to a 1:1 molecular complex, which is devoid of hemolytic activity. This property has been developed into the well known micromethod for the determination of free cholesterol in blood plasma. The same capacity explains how cholesterol combines with some of the plasma proteins and interacts with proteins in cell membranes (Yeagle, 1985).

Hemolysis is one of the many consequences of the action of snake venoms, phospholipases being the responsible enzymes. These esterases act on the serum lecithin, splitting off the hemolytic lysolecithin. It has been found that cholesterol combines in equimolecular proportion with lysolecithin, the product being devoid of hemolytic activity. Ganguly (l937) showed how the rate of hemolysis caused by the Indian cobra (*Naja repudians*) venom in different species of animals is inversely proportional to the cholesterol content of the blood. Totally unaffected are the erythrocytes of sheep, the species with the highest cholesterol content.

4.4. Corticosteroids

Although conclusions are not clearcut, and indeed even controversial, experience with corticosteroids should also be mentioned. These compounds, administered intravenously, met approval in Germany in the 1950's

and early 60's, alone or in combination with antisera. Many encourageing results were reported at that time (Lieske, 1966). No claims were made linking the anti-venom activity to the anti-inflammatory and anti-oedematous action of these steroids. Considering the chemical analogy, the possibility of a protective mechanism analogous to that of the mentioned anti-snake venom steroids should not be dismissed.

4.5. Other steroids

The most famous North American 'snakeroot', *Polygala senega* L., contains α-spinasterol (Simpson, 1937). Other steroids tested by us were tigogenin (40% protection) and hecogenin (20% protection). These are not found in plants reputed active against snake venom, but were shown to have anti-inflammatory properties.

4.6. Pentacyclic triterpenes (free or as glycosides)

Pentacyclic triterpenes with anti-snake venom activity abound. In the following, important examples will be listed, as well as the anti-snake venom plants in which they occur.

Oleanolic acid **6**: *Achyranthes aspera*; *Albizzia lebbeck*; *Allium cepa*; *Calendula officinalis*; *Chiococca alba*; *Forsythia suspensa*; *Marsypianthes chamaedrys*; *Ocimum basilicum*; *Plantago major* ('snakeweed'); *Thymus vulgaris*.

Lupeol (**7** 20% protection): *Aegle marmelos*; *Centipeda minima*; *Crataeva benthami*; *Elephantopus scaber*; *Hemidesmus indicus*; *Marsypianthes chamaedrys*; *Phyllanthus emblica* (2.25%!); *Sophora subprostrata*; *Alstonia boonei*.

Ursolic acid: *Chiococca alba*; *Ehretia buxifolia*; *Marsypianthes chamaedrys*; *Nerium oleander*; *Oldenlandia diffusa*; *Rabdosia amethystoides*; *Thymus vulgaris*.

Taraxerol: *Euphorbia hirta*; *Myrica rubra*; *Taraxacum officinale*.

Taraxasterol: *Calendula officinalis*; *Centipeda minima*; *Taraxacum officinale*.

α-Amyrin: *Chiococca alba*; *Ehretia buxifolia*; *Euphorbia hirta*; *Marsypianthes chamaedrys*; *Alstonia boonei*.

β-Amyrin: *Chiococca alba*; *Byrsonima crassifolia*; *Ehretia buxifolia*; *Euphorbia hirta*; *Marsdenia cundurango*; *Marsypianthes chamaedrys*; *Taraxacum officinale*.

Friedelin (40% protection): *Pentaclethra macroloba*.

Epifriedelinol: *Elephantopus scaber*; *Pentaclethra macroloba*; *Polygonum bistorta*.

Alnusenone: *Polygonum bistorta*.

Betulinic acid (40% protection): *Harpalyce brasiliana*; *Punica granatum*.

Betulin ('betulinol', 40% protection): *Betula utilis*.

Bredemeyeroside (80% protection): *Bredemeyera floribunda*.

Echinocystic acid: *Albizzia lebbeck*; *Chenopodium ambrosioides*.

Cycloartenol: *Linum usitatissimum*.

Quinovic acid (also spelled 'chinovic acid'): *Macfadyena unguis-cati*.

Presenegenin: *Polygala senega*.

Alnusenone: *Polygonum bistorta*.

Gymnemagenin: *Gymnema sylvestre*.

Gypsogenin: *Luffa operculata*.

For many pentacyclic triterpenes, anti-inflammatory and anti-hepatotoxic properties have been reported. Of the above mentioned compounds, oleanolic and ursolic acid are particularly active in this respect. Aminopeptidase *N* activity has been demonstrated for betulinic acid (Melzig and Bormann, 1998).

That triterpenoids and their glycosides possess receptor binding activities has recently been demonstrated. Several compounds of this class, of the *β*-amyrin type, were tested in direct receptor binding assays, as well as to their interaction with a number of specific binding sites. It was equally demonstrated that stigmasterol glucoside binds to serotonin receptors (Zhu and Li, 1999, and earlier papers cited therein).

4.7. Tetracyclic triterpenes

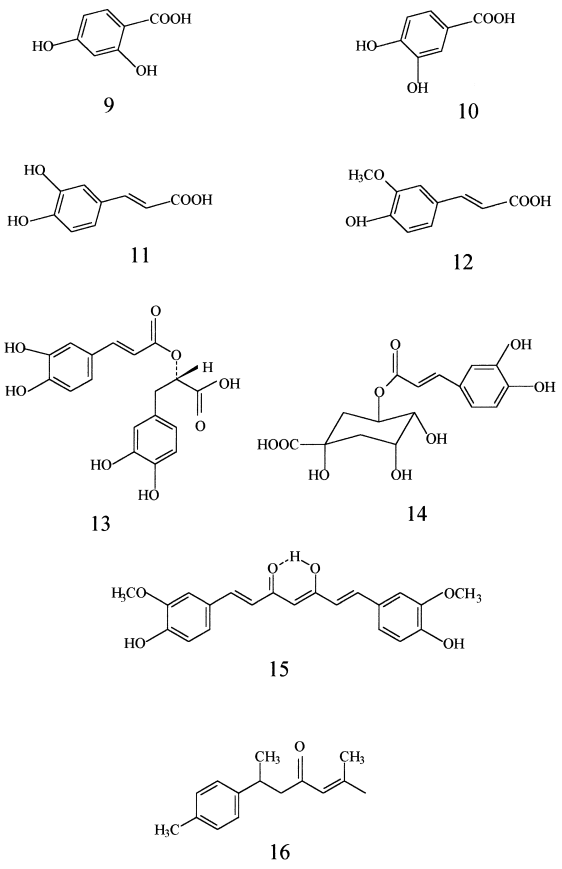
Tetracyclic triterpenes have not been found in anti-snake venom plants. Euphol, also without any indication as to anti-inflammatory activity, proved to be inactive in our essays. It can be concluded that, in the case of anti-snake venom and anti-inflammatory activity, a five-ring structure of a triterpene and corresponding conformation are essential for the completion of the responsible pharmacophore.

4.8. Phenolic compounds

Phenolic compounds are important constituents of anti-snake venom plants. In the present review they will be subdivided into the following categories: hydroxybenzoic acids; cinnamic acid derivatives; coumarins; curcuminoids; flavonoids; and polyphenols (vegetable tannins).

4.9. Hydroxybenzoic acids and their methyl ethers

Of all the benzoic acid derivatives tested by us, 2,4-dihydroxybenzoic acid **9** (83% protection) and 3,4-dihydroxybenzoic acid or protocatechuic acid **10** (80% protection) were the most active. It is remarkable that the 4-*O*-methyl ether of the first was identified as a snake venom neutralizing factor in the Indian anti-snake venom plant *Hemidesmus indicus* (Alam et al., 1994). The corresponding methyl ester, originally described as ''Primula camphor'', along with its glycoside primveroside, occur in several species of the genus *Primula*, *P. denticulata* being another well known anti-



snake venom plant in India. The same compound has been shown to exhibit considerable anti-inflammatory activity (Wagner and Reger, 1987). Equally significant is the fact that the corresponding aldehyde, 2-hydroxy-4-methoxybenzaldehyde, has recently been identified as a potent tyrosinase (polyphenoloxidase) inhibitor (Kubo and Kinst-Hori, 1999), which is not surprising when one considers the strong chelating property of the salicylaldehyde unit.

Protocatechuic acid is equally represented in anti-snake venom plants. Examples are *Fagopyrum cymosum* and *Cryptolepis sinensis*, both from China. In the latter species it was identified as the active constituent (Xiao Peigen, 1981). It is also present in surprisingly high concentration in red-coloured onion skins (*Allium cepa*, already mentioned under cholesterol) and in the northern European 'snakeweed', *Polygonum bistorta*. Vanillin (4-hydroxy-3-methoxy-benzaldehyde) is one of the many constituents identified in *Marsdenia cundurango*, and the corresponding dimethyl ether (veratrum aldehyde) is a component of *Eryngium* species, to which belong some of the 'button snakeroots' of North America. One of these, *E. yuccifolim*, also known as 'rattlesnake master', was official in the US Pharmacopoeia of 1820–1860. Gentisic acid (2,5-dihydroxybenzoic acid) is present in the rhizomes of the yellow gentian, *Gentiana lutea*, an anti-snake venom plant of old employed in many regions of Europe. And 3,4-dihydroxybenzaldehyde (the aldehyde corresponding to protocatechuic acid) has been identified in the rhizomes of the Chinese snake venom antidote *Perilla ternata*.

The monomethyl ether of 2,6-dihydroxybenzoic acid (the free phenol conferring 40% protection) is a component of the bulbs of *Gloriosa superba*, used against snakebite in India; and the methyl ether of *p*-hydroxybenzoic acid (anisic acid) occurs in the North-African anti-snake venom plant *Ruta montana*. *p*-Hydroxybenzoic acid itself, however ('catalpic acid') was inactive in our essays.

Even considering the lack of knowledge with respect to the intricate molecular mechanisms of the action of snake venoms, the capability of many of the substances described to exert agonistic or antagonistic action, will allow for certain conclusions to be drawn from a host of disperse observations (provided, of course, that the compounds themselves, or some equally active metabolites, actually reach their targets). Simple phenols, for instance, can attach to proteins, occupying critical binding sites. Such connections can be effected through hydrogen bonds, strong enough to stabilize the conformation of macromolecules. More stable covalent bonds can also be formed. In plants, phenolic compounds are practically always present in conjugated form; detoxification of simple phenols in plants and animals occurs by conjugation with compounds of the primary metabolism; catechols seize metal atoms by chelation. On the other hand, phenols are substrates for phenolases, resulting in the oxidation to quinones. These, in turn, react with proteins producing covalent linkages. The catechol structure is particularly prone to oxidation to *o*-quinones which condense with proteins resulting in copolymerization (Mason, 1955).

4.10. Cinnamic acid derivatives

Among the cinnamic acid derivatives, caffeic acid **11** and its relatives merit special attention. Other members of the series, like *o*-coumaric and ferulic acid **12**, are also of importance; but in the case of caffeic acid, the chelating property of the catechol moiety and its pronounced character as substrate for phenolases leading to oxidation to quinones confers to this compound exceptional biochemical reactivity.

Caffeic acid itself exhibits a multiplicity of biodynamic activities. To mention just a few: It is a strong lipoxygenase inhibitor, a property for which the catechol structure is also of importance; antihepatotoxic activity has also been demonstrated. Examples of anti-snake venom plants in which caffeic acid is present are several species of *Polygonum*, *Prestonia coalita*, *Strychnos nux-vomica*, *Taraxacum officinale*, as well as a number of plants in which free caffeic acid accompanies chlorogenic acid, where it is present as an ester of quinic

acid. Moreover, the compound, together with ferulic acid, has been identified in considerable,concentration in the oleoresin of several species of pine trees. In east Anatolia (Turkey) the tar of *Pinus sylvestris* is applied externally on the site of snake bites as an antidote to the venom (Sezik et al., 1997). Isoferulic acid is present in another anti-snake venom plant, *Cimicifuga racemosa*, known as 'snake weed', 'snake root' or 'black snake root' in North America.

Caffeic acid is a substituent in many biologically active molecules. Most important examples are the verbascosides, rosmarinic acid **13** and the many caffeic acid derivatives present in *Echinacea species* — plants known since ancient times for their anti-snake venom actitivty by North American Indians. Verbascosides have been identified in several species of *Buddleja* and *Forsythia*, and rosmarinic acid is present in several *Perilla* species. All these botanical genera include anti-snake venom plants. Esters of caffeic acid were identified in species of *Merremia*, a genus also known to contain snake venom antidotes.

Also to be mentioned is the presence of caffeic acid esterified with a triterpene in a non-peptidic antagonist of endothelin: myricerone caffeate, in *Myrica cerifera*. The compound was shown to be a selective antagonist for endothelin receptor, causing specifically the disruption of the endothelin-membrane linkage (Fujimoto et al., 1992).

Several oligomers of caffeic acid (esters between the carboxyl group of one molecule and one or both phenolic hydroxyls of another) were isolated from two African anti-snake venom plants — *Berkleya spekeana* and *Echinops amplexicaulis*. These compounds, as well as the monomeric caffeic acid, proved to be antidotes against snake venoms by oral and parenteral administration (Agoro, 1978).

The receptor activity of caffeic acid has been proved in reecent research on the bioactivity of Argentinian plants, where its ethyl ester was shown to be able to act as competitive ligand for the central benzodiazepine receptors (Marder et al., 1996).

4.11. Chlorogenic acid **14** (60% protection)

Apart from the free acid, caffeic acid occurs mostly esterified with quinic acid, the most widespread product being chlorogenic acid (3-0-caffeyl-D-quinic acid). It exhibits a wealth of biodynamic properties, being inhibitory to a number of enzymes, such as, for instance, lipoxygenase, which explains its antiinflammatory properties. Anti-hepatotoxic activity has also been observed.

This substance has been identified in many anti-snake venom plants, as shown in the following examples: *Achillea millefolium* (yarrow); *Arctium lappa* (burdock, where it occurs together with several other caffeylquinic acids); *Citrullus colocynthis*; *Coffea arabica* (coffee seeds and leaves); *Fagopyrum cymosum*; *Helianthus annuus* (sunflower); *Marsdenia cundurango*; *Nicotiana tabacum*; several *Strychnos* species, mainly *S. colubrina* ('lignum colobrinum' or 'snake wood'); and *Polygonum bistorta* (European snakeweed). In the above mentioned *Strychnos* species the compound was originally described as 'igasuric acid'.

The capacity of caffeic acid and chlorogenic acid to bind to proteins has been investigatred in depth with human serum albumin (Muralidhara and Prakash, 1995). Strong interaction between these molecules has been shown to occur, with consequent conformational change in the protein. Caffeic acid interacts even more strongly than chlorogenic acid.

4.12. Other phenylpropanoid derivatives

Compounds in which two caffeic acid units are esterified with quinic acid are known to occur in plants, but are relatively rare. Several of them have been identified in yarrow (*Achillea millefolium*) and in species of *Artemisia* (Kimura et al., l985; Okuda et al., 1986), plants which have a reputation for activity against the action of snake venom. Cynarin (1,5-dicaffeoylquinic acid, 20% protection), produced in artichoke leaves as an artifact of transesterification during work-up. is held responsible for the choleretic and anti-hepatotoxic activities of this drug, which is popular in cholagogue remedies, but unknown as an anti-snake venom plant. Even so, extracts are sometimes recommended against snake poison envenomation, reportedly with success. Esters of various hydroxycinnamic acids with glucose occur in *Polygala senega* (Corner et al., 1962).

4.13. Curcuminoids

Three diarylheptanoids — curcumin **15** (diferuylmethane), demethoxycurcumin and bis demethoxycurcumin- make up the yellow dye of the rhizomes of turmeric, *Curcuma longa*, and other *Curcuma* species. These 'curcuminoids' are the only natural pigments of this class. Well known as a spice and coloring matter, turmeric is also widely used as medicinal in oriental tradition, the treatment of snake bite poisoning being one of its uses. Intestinal absorption of curcumin is about 60–65% (Srimal, 1997). In the first scientific work on the subject, the extract of the plant was shown to inactivate almost completely the neurotoxin of the cobra, *Naja naja siamensis* (Cherdchu and Karlsson, 1983 and bibliography cited therein).

In the structure of curcumin, two ferulic acid moieties are linked via a methylene bridge, resulting in a conjugated diketone which, in solution, through keto-enol tautomerism, produces a strong chelating centre. Many elements have been shown to form chelates with the curcuminoids. Reaction with boric acid has been known

since 1866. At present, practical use for this property is in the spectrophotometric determianation of boron.

There are indications that curcumin also interacts strongly with biological macromolecules, like serum proteins, albumin and hyaluronic acid (Tonnesen, 1992). Among the many pharmacological properties shown by the curcuminoids, anti-inflammatory, hepatoprotective, anti-mutagenic, anti-carcinogenic, inhibition of lipoxygenase and prostaglandin-endoperoxide synthase stand out (Kiso et al., 1983; Amman and Wahl, 1990).

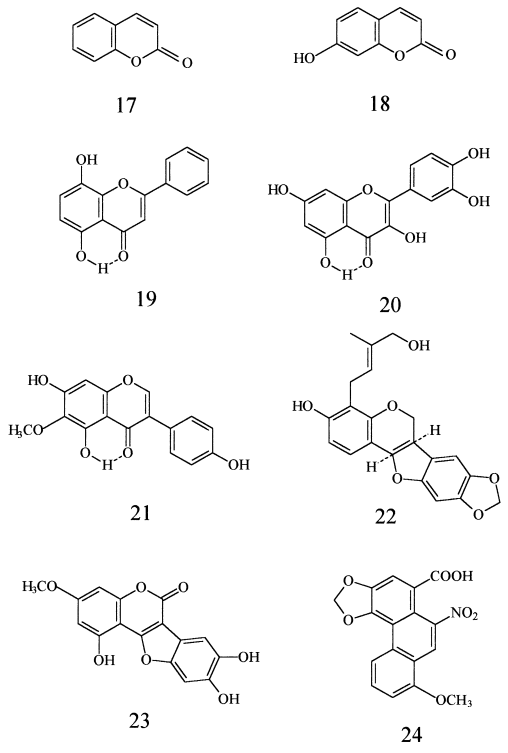
Another unsaturated ketone of turmeric, ar-turmerone **16**, was shown to inhibit the lethal action of rattlesnake venom, in addition to exhibiting other important biological activities (Ferreira et al., 1992).

4.14. Coumarins

Coumarin **17** (40% protection), the simplest representative of its class, occurs often in considerable amounts in anti-snake venom plants. Examples are: *Dipteryx odorata* (the 'tonka bean') and *D. punctata*; *Liatris squarrosa*; several *Mikania* species; and *Torresea cearensis*.

All other coumarins are oxygenated at C-7, being therefore derivatives of umbelliferone **18**, which occurs in *Aegle marmelos*, *Daphne mezereum* and *Ipomoea batatas*. This latter species produces also scopoletin, abundant as well in several species of *Brunfelsia* and in *Heterothalamus psiadioides*, here accompanied by its dimethyl ether, scoparone. Herniarin (7-methoxycoumarin) and ayapin (6,7- methylenedioxy-coumarin), isolated from the Amazonian anti-snake venom plant *Eupatorium triplinerve*, were shown to exhibit considerable hemostatic activity, a property not necessarily shared by the other coumarins (Bose and Sen, 1941). An exceptionally high content of daphnin, the 7-*O*-glucoside of daphnetin (7,8-dihydroxycoumarin) accompanies umbelliferone in the leaves of *Daphne odora* (up to 27% on the dry weight of the leaves, depending on their age (Asai, 1930). Suberenone is present in *Ruta graveolens*; marmin in *Aegle marmelos*. *Dorstenia brasiliensis* and *Feronia limonia* contain several furano-coumarins, among them bergapten (20% protection) and the monoterpenoid substituted dorstenin (Kuster et al., 1994) and fernolin (Agrawal et al., 1989). All the mentioned species are highly reputed remedies against snake bite.

Considering the great quantity of coumarins occurring in the plant kingdom, and the varied effects they produce on plants and animals, one would expect to find them in many more anti-snake venom plants; but they are conspicuous only in the above mentioned ones. In spite of much research conducted in recent years (Hoult and Payá, 1996; O'Kennedy and Thornes, 1997), not much is known about their mechanism of action. One still has to agree with a statement made over twenty



years ago by Stewart A. Brown: "Remarkably little is understood about the mechanism of action at the molecular level. A number of investigations in plants have demonstrated inhibition by coumarin of various enzymes. Nevertheless there appears to be agreement that the primary effect of coumarins underlying most of the phenomena attributed to their action remains unknown" (Brown, 1977).

4.15. Flavonoids

Of all the secondary metabolites capable of complex formation, the flavonoids are probably the most versatile. These compounds embrace a wealth of possibilities of hydrogen bonding arranged around a relatively small carbon skeleton, capable of interacting with molecular targets. Grassmann et al. (1956) demonstrated the considerable strength of the hydrogen bonds between phenolic hydroxyl groups and the amides of protein chains; Dinya and Hetenyi (1975) suggested a model for the localization of the different forces involved in a flavonoid-receptor, complex; and Jin et al. (1990), on determining the crystal structure of rutin by X-ray diffraction, were able to show how all four free hydroxyl groups of the molecule participate in hydrogen bonds and, along with the carbonyl group of the pyrone ring, are spatially fixed and liable to occupy the active site of a receptor.

It is, therefore, very plausible to understand the extraordinary widespread biodynamic activities of these compounds as a consequenece of their ability of binding

to biological polymers. In fact, flavonoids have been held responsible for anti-inflammatory, anti-hepatotoxic, anti-hypertensive, anti-arrhytmic, hypocholesterolemic, anti-allergic, antitumour and many other activities and, most important in the context of our subject, enzyme inhibiting activity. Flavonoids have been shown to inhibit phospholipases A_2, important components of snake venoms (Alcaraz and Holt, 1985); and quercetin is a potent inhibitor of lipoxygenase.

This enzyme inhibiting property has long been recognized, to the point where flavonoids have to be removed, for instance, with the aid of borate buffers, when studying certain enzymatic activities in plant material. It is due mainly to their affinity to peptides (enzymes and their substrates), and also to their metal binding capacity, a property which has application even in analytical chemistry. Thus, morin, present in the anti-scorpion sting plant *Artocarpus integrifolia* and in the anti-snake venom plant *Morus alba*, finds use as a reagent for aluminium and zinc, among other metals. And several snake venom enzymes are zinc-containing metalloproteinases (Bjarnason and Fox, 1994).

The subject of biological properties of flavonoids has been amply reviewed since the early eighties (Havsteen, 1983) and in more depth and more expansion ever since. It is beyond the scope of this article to condense the multitude of information by now available.

Some of the simplest flavonoids can be found in the genus *Primula*. A good example is primetin **19** (5,8-dihydoxyflavone), a constituent of the Indian anti-snake venom plant *Primula denticulata*, where it occurs in a farinaceous floury coating, a powerful contact allergen which covers these plants (Harborne, 1971).

Quercetin and several of its glycosides are the flavonoids most often encountered in anti-snake venom plants. Good examples are the following:

Free quercetin **20** (40–80% protection) in *Albizzia lebbeck* and in onion skins (*Allium cepa*); rutin (quercetin 3-0-rutoside, 20–80% protection) in *Achillea millefolium, Euphorbia hirta, Forsythia suspensa, Marsypianthes chamaedrys, Nerium oleander* and *Ruta graveolens*; other quercetin glycosides in *Foeniculum vulgare, Elianthus annuus, Nerium oleander, Polygonum bistorta* and *Rheum palmatum*.

Interestingly, already fifty years ago, a Brazilian scientist, Roched A. Seba, working at Instituto Vital Brazil, described the protective action of rutin, applied together with an anti-histamine, against vascular effects caused by *Bothrops atrox* venom (Seba, 1949).

Other flavonoids and anti-snake venom plants in which they occur, either free or as glycosides, can be cited:

Hesperetin in *Prunus persica*; pinostrobin in *Heterothalamus psiadioides*; naringenin in *Prunus persica*; galangin in *Heterothalamus psiadioides*; apigenin (40% protection) in *Boehmeria nivea*; kaempferol in *Cassia tora*, *Impatiens capensis*, *Nerium oleander*, *Paeonia albiflora* and *Prunus persica*; luteolin in *Ajuga decumbens* and *Merremia tridentata*; diosmetin in *Merremia tridentata*; isorhamnetin in *Argemone mexicana,* morin in *Morus alba; myricetin in Myrica rubra. Phyllanthus niruri* and *P.urinaria* contain quercetin and its glycosides rutin, quercitrin and isoquercitrin.

The presence of flavonoids becomes particularly conspicuous where the flowers are the reputed active part of the plant, as in *Calendula officinalis* (isorhamnetin), *Hibiscus mutabilis* (quercetin and several others), *Impatiens balsamina* (myricitin) and *Lonicera japonica* (luteolin).

All the mentioned flavonoids, without exception, show the structural feature of the proximity and coplanarity of the phenolic hydroxyl group on carbon atom 5 and the pyronic carbonyl, already alluded to above. The same characteristic is present in the chromones isolated from the African *Schumannophyton magnificum*, the bark of which has the reputation of a snake venom antidote (Houghton, 1988 and references cited therein). Moreover, Lindahl and Tagesson (1997) described the inhibitory effect of flavonoids — particularly rutin — on phospholipase from several species of snake. They, too, stressed the importance of the mentioned structural feature for this inhibition.

The mentioned biodynamic properies are intrinsic to the flavonoids themselves. Flavonoids, however, when ingested by humans, are degraded by action of the intestinal microflora, producing metabolites which are in turn active. The first step in this degradation sequence is the hydrolysis of glycosides. The transformation continues by fission of the pyrone ring, producing smaller molecules — phenolic acids — some of which already mentioned above as active against snake venom. These were detected in the blood serum and urine of individuals, after oral administration of flavonoids. In this way it was shown that protocatechuic acid, for instance, is a metabolite of quercetin. In some of the assays for biological activity the metabolites were even more effective than the parent flavonoids (Gross et al., 1996; Kim et al., 1998; Graefe and Veit, 1999; Han, 1998).

Evolutionarily advanced flavonoids:isoflavonoids, pterocarpans and coumestans.

Isoflavonoids as a group show many biodynamic properties. Even so, they are not frequent in anti-snake venom plants. A few should be mentioned. Tectoridin, the 7-*O*-glucoside of tectorigenin **21**, and iridin, the 7-*O*-glucoside of irigenin, make up 1.5% of the rhizome and roots of the iridaceae *Belamcanda chinensis*, highly reputed as a venom antidote in China. Again, both these compounds are quoted as antiinflammatory and hepatoprotective.

Another isoflavonoid, derricidin, isolated by us from the roots of *Derris sericea* (not used as a venom antidote) showed in our assays 70% protection.

4.16. Pterocarpans

The isolation of two prenylated pterocarpans provided the impulse for the more recent interest in snake venom antidotes: cabenegrins A-I **22** and A-II (Nakagawa et al., 1982). The compounds were identified in the anti-snake venom remedy 'Específico Pessoa', a plant extract produced and sold in the northeast of Brazil, 100% active in our essays. More recently, a similar compound, which was called edunol, was isolated from the Mexican anti-snake venom plant *Brongniartia podalyrioides* and shown to neutralize the lethal action of the venom of *Bothrops atrox* (Reyes-Chilpa et al., 1994).

Many other prenylated pterocarpans found to be bioactive were shown to occur in plants of the genus *Erythrina* (Mitscher et al., 1987). Of these, the bark of *E.barteroana* is known as an anti-snake venom plant in Guatemala.

Curiously, the botanical identity of, the plant which furnishes 'Específico Pessoa' has never been ascertained, and is not even mentioned in the corresponding patent application. The identification of still other, similar, prenylated pterocarpans from the roots of another northeastern Brazilian snake venom antidote, *Harpalyce brasiliana*, makes it probable that this is the parent plant of 'Específico Pessoa' (Silva et al., 1997).

4.17. Coumestans

It was with *Eclipta prostrata* that our group started to study snake poison antidotes over ten years ago. The plant is well known as an anti-snake poison both in China and in Brazil. In China the same species is also used as anti-hepatotoxic. Several compounds — flavonoids, phytosterols, and one coumestan, wedelolactone- were identified in the extracts of the plant by different authors. Among the components,wedelolactone **23** (40% protection), stigma sterol, and sitosterol were found to be the main ones capable of neutralizing the lethal effect, on mice, of South American rattlesnake (*Crotalus durissus terrificus*) venom (Mors et al., 1989)

Wedelolactone was shown to exert several well defined pharmacological actions: antimyotoxic, antihemorrhagic, antiproteolytic, antiphospholipasic (Melo et al.,1989, 1993, 1994). Also wedelolactone and another coumestan from the same plant — demethylwedelolactone — were identified as the main active antihepatotoxic constituents (Wagner et al., 1986).

4.18. Aristolochic acids

The roots of Aristolochia species are famous remedies against snakebite. The names 'Virginia snakeroot'and 'serpentary' (*A. serpentaria*) allude to this reputation. Most of the chemically studied *Aristolochia* species produce in their roots peculiar organic nitro-compounds, having a phenanthrene nucleus: the aristolochic acids and aristolactams. The interaction of these (probably the most abundant aristolochic acid I **24**) with oedema-inducing enzymes from Indian Viperidae has been studied in detail (Tsai et al., 1980). With the aid of a circular dichroism study, Vishwanath et al. (1987) found that aristolochic acid forms a 1:1 complex with phospholipase A_2, acting like a non-competitive inhibitor of the enzyme. The chelating properties of nitro-compounds with the nitro-group in appropriate vicinity to an acidic hydrogen atom, producing an intramolecular hydrogen bridge, are well known. From their observations with the aid of circular dichroism and spectral observations in the near ultraviolet, the Indian authors concluded that the aristolochic acid-enzyme association causes a significant change in the secondary structure of the protein.

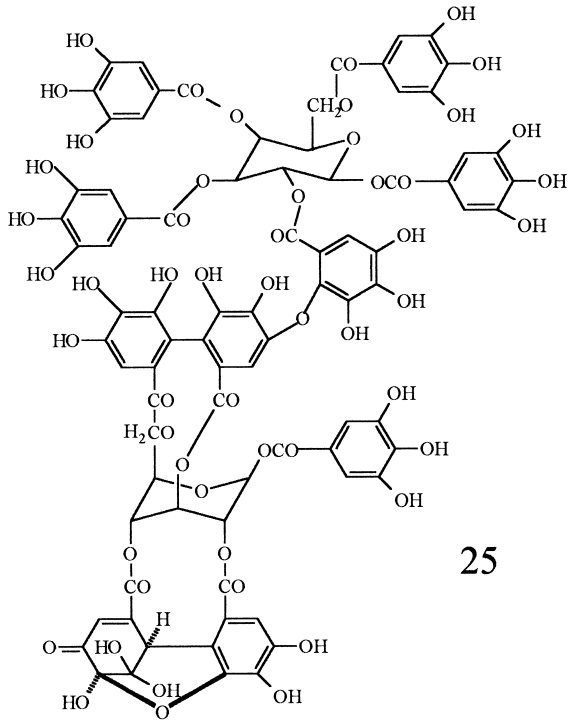
4.19. Vegetable tannins

The first scientific approach to a natural anti-snake venom remedy, in modern times, concerns not a micromolecule but a much larger polyphenol. Japanese workers reported about the detoxifying action of persimmon tannin (from the unripe fruits of *Diospyros kaki*) as an acknowledged medicine against snake venom envenomation, in Japan (Okonogi and Hattori, 1978; Okonogi et al., 1979). The chemical structure of this tannin was studied by Matsuo and Ito (1979). Two ellagitannins from *Euphorbia hirta*, Eufhorbins A **25** and B had their complicated structures elucidated (Yoshida et al., 1988).

Less well investigated are many other tanniferous plants used to the same effect, among them several species of *Acacia* (e.g. *A. catechu*, *A. hindsi*, *A. leucophloea*, *A. nubica*, *A. polyacantha* and *A. sinuata*); the root of rhubarb (*Rheum palmatum*); and the leaves of sumac (*Rhus semialata*), used for tanning. Interesting in this repect, also, is the use of the juice of the stem and rootstock of the banana tree (*Musa paradisiaca*), used against snake bite in the Caribbean area.

Among the mechanisms of action of all the compounds considered, the one of the vegetable tannins is probably the best understood, due to the exhaustive work at Sheffield about the chemical nature of protein-tannin interaction (Haslam, 1989). Also, the enzyme inhibiting activity of tannins are well known — a property which they share with many of the micromolecular compounds mentioned in this article.

The action of these compounds as drugs is exposed in another article (Haslam, 1996). Most remarkably, of the seven botanical genera mentioned there as examples of tannin containing medicinal plants, three include well known anti-snake venom species, viz., *Paeonia*, *Agrimonia* and *Rubus*.



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4.20. Polysaccharides

Other, heavier, constituents can be counted among biolgically active entities. Polysaccharides have been shown to exhibit mainly antiinflammatory and immunomodulating activities. These properties can also be extended to anti-snake venom actions.

The bark of *Casearia sylvestris* is known as such a remedy throughout Brazil. Its aqueous extract (85% protection) yielded, besides sitosterol and stigmasterol, a mixture of polysaccharides (30% protection), which could be resolved into five distinct units, three being neutral and two of acidic nature (Barbi, 1992). *Calendula officinalis*, an anti-snake venom plant already mentioned to contain steroids and triterpenes, has its wound healing action attributed to the presence of an immunostimulating heteroglycan (Varlen et al., 1989), which could also well be responsible for antiophidic activity.

4.21. Concluding remarks

It was shown how chemical constituents — the so-called 'secondary metabolites' — can be held responsible for the neutralizing effect of plants against the action of snake venoms, in popular use. The evidence is overwhelming. The many different chemical structures shown to occur in such plants are all capable of interacting with macromolecular targets. Snake venoms are of a highly complex nature, made up of peptides and proteins. Many of these components are enzymes. The mechanisms of their actions, still incompletely understood (Dufton and Hider, 1977, 1980), can in great part be attributed to the blocking of receptors — structures prone to chemical attack. Other vulnerable sites are metal atoms — notably zinc — present in metallo-proteinases, where sequestering by chelation offers a plausible explanation for the inhibition of enzymes (e.g. Bush et al., 1984). Endogenous peptides, for instance, can inhibit metalloproteinases in *Bothrops asper* venom (Francis and Kaiser, 1993). Thus, the organisms which are victims, as well as the venoms themselves, offer a multiplicity of binding sites (Menez, 1985).

In reviewing the above exposure one comes to the conclusion that many of the plants recorded as anti-snake venoms in popular use just *must* have antidotal properties, due to the great number of active compounds they contain. A few examples should suffice to validate this point. For instance: the leaves of *Citrullus colocynthis* contain sitosterol, spinasterol, caffeic acid, ferulic acid, chlorogenic acid, quercetin, and rutin; *Eclipta prostrata* contains sitosterol, stigmasterol, β-amyrin, apigenin, luteolin, wedelolactone, wedelic acid and demethylwedelolactone. *Euphorbia hirta* contains sitosterol, stigmasterol, campesterol, cycloartenol, friedelin, α-amyrin, β-amyrin, taraxerol, taraxerone, ellagitannins and the flavonoids rhamnetin and quercetin and their glucosides; the root of *Fagopyrun cymosum* contains protocatechuic acid, *p*-coumaric acid, ferulic acid, quercetin and several of its glucosides; *Marsdenia cundurango* contains β-amyrin, vanillin, *p*-coumaric acid, caffeic acid, chlorogenic acid, neochlorogenic acid, coumarin, umbelliferone, esculetin, and several flavonoids; *Marsypianthes chamaedrys* contains sitosterol, stigmasterol, ursolic acid, oleanolic acid, tormentic acid, lupeol, germanicol, α-amyrin, β-amyrin and rutin.

Considering all the facts, obviously many plants which meet the necessary conditions were *not* discovered by the people. We can quote from our own experience: The aqueous extract of wood and bark of the tree *Apuleia leiocarpa* showed 100% protection towards jararaca venom after 24 h (Gonçalves et al., 1991). The plant material used in these tests contains sitosterol, β-amyrin and several flavones (Braz-Filho and Gottlieb, 1971). And *Phyllanthus klotzschianus*, a plant which also gives 100% protection, contains sitosterol glucoside, protocatechuic acid, quercetin and rutin, among several other identified constituents (Kuster, 1994). *Alpinia speciosa*, rich in kaempferol, also confers 100% protection.

Exogenous natural micromolecules can mimic the biological activity of endogenous macromolecules. Striking examples are the opium alkaloids, which bind to opioid receptors and thus simulate the action of endogenous opioids with morphine like activity — the endorphins.

Closer to the point in the present article is the action of myricerone caffeate, mentioned above under the cinnamic acid derivatives. This substance, a caffeic acid

ester of a triterpenoid, isolated from bayberry, is an endothelin antagonist, reproducing the endothelin blocking activity of several identified peptides (Fujimoto et al., 1992).

Coming back to anti-snake venom activity, wedelolactone, a coumestan fom the anti-snake venom plant *Eclipta prostrata*, for which many biodynamic activities have been demonstrated, can antagonize different types of phospholipase A_2 myotoxins (Melo and Ownby, 1999).

Generalizing, it can be inferred that micromolecules which neutralize the action of snake venoms mechanistically replace endogenous antitoxic serum proteins with neutralizing capacity such as produced by some animals (Perez et al., 1978; Thwin and Gopalkrishnakone, 1998) — a subject well studied in the case of the immunity of the opossum (*Didelphis marsupiales*) towards the venom of the jararaca (*Bothrops jararaca*) snake (Moussatché et al., 1979; Perales et al., 1986; Domont et al., 1991).

Acknowledgements

We are grateful to Dr. Anibal Melgarejo of Instituto Vital Brazil for generous supply of jararaca venom. Decisive collaboration in this project by many colleagues, especially Paulo de Assis Melo, Ricardo Machado Kuster, José Paz Parente, Robson Ronei Bernardo and Maria Helena da Silva, is gratefully appreciated. Thanks are also due to Ms. Franca Milione for important aid in the survey of the literature; and to Mr. Natanael Rabelo Gonçalves for excellent technical assistance. Financial support was received from the Brazilian National Research Council, CNPq; the Coordination of Post-Graduate Activities, CAPES; the Foundation for the Support of Research in the State of Rio de Janeiro, FAPERJ; and the Federal University of Rio de Janeiro, UFRJ.

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