CHAPTER 18

Tranquilizing Medicinal Plants:
Their CNS Effects and Active Constituents—Our Experience

Mariel Marder and Cristina Wasowski

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INTRODUCTION

Mental disorder is a psychological or behavioral pattern that occurs in an individual and is thought to cause distress or disability that is not expected as part of normal development or culture. Mental and neurological disorders are highly prevalent worldwide, with 450 million people estimated to be suffering from them. They are responsible for about 1% of deaths, and they account for almost 11% of disease burden the world over. The magnitude of neurological disorders is huge, and these disorders are priority health problems around the world. The extension of life expectancy and the aging of the general populations in both developed and still-developing countries are likely to increase the prevalence of many chronic and progressive physical and mental conditions, including neurological disorders. The proportionate share of the total global burden of disease attributable to neuropsychiatric disorders is projected to rise to 14.7% by 2020 (WHO).

There are many different categories of mental disorders and many different facets of human behavior and personality that can become disordered. Mental illnesses are classified according to the symptoms that a patient experiences, as well as the clinical features of the illness. Some of the major categories of mental illness include anxiety disorders, cognitive disorders, developmental disorders, dissociative disorders, mood disorders, personality disorders, schizophrenia, and substance abuse disorders.

“Anxiety” is defined as a subjective emotional state of uneasiness, not pleasant, and even fearful. When the anxiety reaches pathological levels, the subject experiences conductual changes, apprehension, motor troubles, sweating, and hypertension.

The term “sedation” implies a general slowing down of cognitive functioning, whereas a “hypnotic” specifically means the induction of sleep itself. Conversely, “tranquilization” signifies emotional calming that may or may not lead to sleep but does not induce the feeling of drowsiness.

Traditional medicine has many cures for these ailments, most of them based on herbal preparations; however, modern medicinal chemistry has provided several drugs that are more or less effective, for the same purpose. The most spectacular success was achieved in 1957 with the synthesis of the benzodiazepines [Sternbach 1978], which still are, after 50 years of intense clinical research and use, the near-best medication to treat mental disorders.

Benzodiazepines, however, also produce several side effects, such as sedation, muscle relaxation, alcohol incompatibility, amnesia, and addiction [Woods et al. 1992]. These drawbacks have to be carefully considered in clinical therapeutical applications.

Although benzodiazepines are laboratory products, they were found also in nature, and, appropriately, their first detection was in the mammalian brain [Sangameswaran et al. 1986]. They were then identified in many other sources, such as foods, rumen, plasma, and cow’s and human’s milk [Medina and Paladini 1993].

When we attempted detection of benzodiazepines in several plants, including some used to prepare tranquilizing infusions, we unexpectedly discovered that some flavonoids present in them were ligands for the benzodiazepine binding site of the...
gamma aminobutyric acid receptor type A (GABA\textsubscript{A}) [Medina et al. 1989, 1997, 1998; Paladini et al., 1999; Marder and Paladini 2002].

A search for novel pharmacotherapy from medicinal plants for psychiatric illnesses has progressed significantly in the past 20 years. This is reflected in the large number of herbal preparations for which psychotherapeutic potential has been evaluated in a variety of animal models. A considerable number of herbal constituents, whose behavioral effects and pharmacological actions have been well characterized, may be good candidates for additional investigations that may ultimately result in clinical use. Herbal remedies that have demonstrable psychotherapeutic activities have provided a potential to psychiatric pharmaceuticals and deserve increased attention in future studies.

This chapter deals with plants possessing CNS effects. However, because of the huge amount of plants belonging to this category, we decided to select a few plants and to focus our attention on them, mostly concerning the constituents that have significant therapeutic effects in animal models of CNS disorders.

**ANIMAL MODELS**

An animal model is a nonhuman animal that has a disease or injury that is similar to a human condition. These test conditions are often termed as animal models of disease. The use of animal models allows researchers to investigate disease states in ways that would be inaccessible in a human patient, performing procedures on the nonhuman animal that imply a level of harm that would not be considered ethical to inflict on a human.

To serve as a useful model, a modeled disease must be similar in etiology (mechanism of cause) and function to the human equivalent. Animal models are used to learn more about a disease, its diagnosis, and its treatment. For instance, behavioral analogs of anxiety or pain in laboratory animals can be used to screen and test new drugs for the treatment of these conditions in humans.

Housing, handling, and experimental procedures complied with the recommendations set forth by national and international committees for the care and use of laboratory animals, and all efforts are taken to minimize animal suffering. The number of animals used is always the minimum number consistent with obtaining significant data.

**Radioreceptor Binding Assays (In Vitro)**

Radioligand binding assays are used to evaluate the putative action of the extracts or their constituents on different brain receptors in brain homogenates (bovine, rat, and mouse).

**Pharmacological Studies Conducted on Mice (In Vivo)**

Figure 18.1 shows the pharmacological assays used in our laboratories to assess behavior in mice.
The Hole-Board Assay

The hole-board test, which was first introduced by Boissier and Simon [1962, 1964], offers a simple method for measuring the response of an animal to an unfamiliar environment. Previously, the hole-board test has been used to assess emotionality, anxiety, and/or responses to stress in animals [Rodriguez Echandia et al. 1987]. Some advantages of this test are that several behaviors can be readily observed and quantified, which makes possible a comprehensive description of the animals’ behavior.

This assay is conducted in a walled, black Plexiglas arena with a floor of approximately 60 × 60 cm and 30-cm-high walls, with four centered and equally spaced holes...
in the floor, 2 cm in diameter each and illuminated by an indirect and dim light. Each animal, after the intraperitoneal injection of the vehicle or the drug, is placed in the center of the hole-board and allowed to freely explore the apparatus for 5 min; then, the number of holes explored, the time spent head dipping, and the number of rearings are measured. Changes in head-dipping behavior in the hole-board test reflect the anxiogenic and/or anxiolytic state in mice [Kliethermes and Crabbe 2006].

Assessment of Locomotor Activity

The spontaneous locomotion activity is measured in a box made of Plexiglas, with a floor of $30 \times 15$ cm and 15-cm-high walls. The locomotor activity is measured and expressed as total light beam counts during 5 min.

Sodium Thiopental-Induced Loss of Righting Reflex

A subhypnotic dose of sodium thiopental (35 mg/kg) is intraperitoneally injected to mice 20 min after a similar injection of the vehicle or the drug. The time of loss of righting reflex is determined as the interval between the loss and the recovery of the reflex [Ferrini et al. 1974]. The disappearance and the reappearance of the righting reflex are considered indications of duration of sleep.

The Elevated Plus-Maze Test

This test is based on the natural aversion of rodents for open spaces and uses a maze with two open and two closed arms ($25 \times 5$ cm, each), with free access to all arms from the crossing point. The closed arms had walls 15 cm high all around. The maze is suspended 50 cm from the room floor. After administration of the drugs, mice are placed on the central part of the cross, facing an open arm. The number of entries and the time spent going into open and closed arms were counted during 5 min under red, dim light. A selective increase in the parameters corresponding to open arms reveals an anxiolytic effect. The total exploratory activity (number of entries in both arms) is also determined [Pellow et al. 1985].

Light/Dark Transition Test

The model is based on the innate aversion of rodents to brightly illuminated areas and on the spontaneous exploratory behavior in response to novel environment and light. The light/dark box consists of a Plexiglas box monitored with two compartments, distinguished by wall color, illumination, and size; one light area ($30 \times 21 \times 21$ cm, length $\times$ width $\times$ height) illuminated by a 60 W light in the ceiling of the compartment and with white walls, and a smaller dark compartment ($14 \times 21 \times 21$ cm, length $\times$ width $\times$ height) with black walls and not illuminated. An opening door ($6 \times 3$ cm) located in the center of the partition at floor level connected the two compartments.
Animals are placed in the center of the dark or white area facing the wall opposite to the door. The following parameters were recorded during 5 min: (1) latency time of the first crossing to the light compartment, (2) the number of crossings between both compartments, (3) the total time spent in the illuminated zone of the cage, and (4) the overall movements in both areas [Bourin and Hascoët 2003]. A selective increase in the parameters corresponding to the light compartment and transitions reveals an anxiolytic effect.

**Horizontal Wire Test**

This assay is performed to evaluate myorelaxant effects of the drugs. The mice are lifted by the tail and allowed to grasp a horizontally strung wire (1 mm diameter, 15 cm long, and placed 20 cm above the table) with their forepaws, after which they are then released. The number of mice from each treatment group that do not grasp the wire with their forepaws within a 5 s period is recorded. A myorelaxant drug would impair the ability of the mice to grasp the wire. Muscle relaxation is commonly associated with sedation [Bonetti et al. 1982].

**Seizure Testing**

The pentylenetetrazol (PTZ) test is one of the models that represent the in vivo system most commonly used in the search for effective antiepileptic drugs.

In this assay, PTZ (200 mg/kg) is administered intraperitoneally to mice 15 min after injection of drug or vehicle. The number of mice showing clonic or tonic-clonic convulsions is determined.

**SOME HERBS USED IN FOLKLORIC MEDICINE AS TRANQUILIZERS**

Anxiolytics and sedatives essentially have the same underlying mechanisms of action: the stronger the agent, the greater the sedative effect, leading to coma in extreme cases. Four mechanisms of action have been implicated: (1) binding to GABA receptors leading to hyperpolarization of the cell membrane through increased influx of chlorine anions; (2) inhibition of excitatory amino acids, thereby also impairing the ability to form new memories; (3) sodium channel blockade, decreasing depolarization of the cell membrane; and (4) calcium channel blockade, decreasing the release of neurotransmitters into the synaptic cleft. Most complementary medicines prescribed for anxiolysis/sedation (e.g., kava kava, valerian, passion flower, and chamomile) could be GABAergic, although for some the mechanism of action remains unknown [Werneke et al. 2006].

**Passiflora Species (Passion Flower)**

The genus *Passiflora*, comprising about 500 species, is the largest in the family Passifloraceae (the passion flower family). The species of this genus are distributed
in the warm temperate and tropical regions of America; they are much rarer in Asia, Australia, and tropical Africa. Several species are grown in the tropics for their edible fruits; the most widely grown is the *Passiflora edulis* Sims (passion fruit or purple granadilla). The discovery of several thousand year old seeds of *Passiflora* from the archaeological sites at Virginia and North America provides strong evidences of the prehistoric use of the fruits by the ancient “Red Indian” people.

The use of *Passiflora* as a medicine was lauded for the first time by a Spanish researcher Monardus in Peru in 1569, because the beautiful flowers of *Passiflora* appeared to him to be symbolic of the passion of Christ. Various species of *Passiflora* have been used extensively in the traditional system of therapeutics in many countries.

The extract of *Passiflora alata* (fragrant granadilla), with aloes, was reputed beneficial in atrophy of various parts. In Brazil, the said species, known as “Maracuja,” has been put to use as an anxiolytic, sedative, diuretic, and an analgesic.

*Passiflora capsularis* is a reputed emmenagogue. *Passiflora contrayerva* is a counter-poison, deobstruent and cordial. *Passiflora edulis*, sometimes known as the “passion fruit,” has been used as a sedative, diuretic, anthelmintic, antidiarrheal, stimulant, tonic, and also in the treatment of hypertension, menopausal symptoms, and colic of infants in South America. *Passiflora foetida* leaf infusion has been used to treat hysteria and insomnia in Nigeria. This plant is widely cultivated in India. The leaves are applied on the head for giddiness and headache; a decoction is given in biliousness and asthma.

The *Materia Medica Americana*, a Latin work published in Germany in 1787, mentions the use of *Passiflora incarnata* to treat epilepsy of the aged. An ancient report describes the use of this plant in spasmodic disorders and insomnia of infants and the old. *Passiflora incarnata* is a popular traditional European remedy as well as a homeopathic medicine for insomnia and anxiety and has been used as a sedative tea in North America. The plant has been used (1) as an analgesic, antispasmodic, antiasthmatic, wormicidal, and sedative in Brazil, (2) as a sedative and narcotic in Iraq, (3) in diseased conditions such as dysmenorrhea, epilepsy, insomnia, neurosis, and neuralgia in Turkey, (4) to cure hysteria and neurasthenia in Poland, and (5) to treat diarrhea, dysmenorrhea, neuralgia, burns, hemorrhoids, and insomnia in America.

*Passiflora laurifolia* Linn. (yellow granadilla, Jamaica honeysuckle) is used to treat nervous heart palpitations in Trinidad. The juice of *Passiflora maliformis* Linn. is used for intermittent fevers in Brazil. *Passiflora quadrangularis* Linn. (giant granadilla) is used throughout the Caribbean as a sedative and for headaches [Dhawan et al. 2004].

*Passiflora coerulea* L.

*Passiflora coerulea* (blue Passion flower), native to Brazil and introduced into Britain in the 17th century, is the most vigorous and tender species having traditional use of its fruit as a sedative and anxiolytic. In the West Indies, Mexico, the Netherlands, and South America, the root has been used as a sedative and vermifuge. In Italy, the plant has been used as an antispasmodic and sedative. In Mauritius,
tincture and an extract of the plant had been used as a remedy for insomnia caused by various nervous conditions but not caused by pain. The root has been used as a diuretic and a decoction of leaf as an emetic. In Argentina folk medicine, the aerial parts of *Passiflora coerulea* (where it is known as the Pasionaria or Mburucuyá in Guaraní) are used as mild antimicrobial agents in diseases, such as catarrh and pneumonia, and as a sedative.

Chrysin, 5,7-dihydroxyflavone (Figure 18.2), was isolated and identified from the dried branchlets of *Passiflora coerulea* L. (Passifloraceae). Chrysin was found to be a ligand for the benzodiazepine binding site in the GABA<sub>A</sub> receptor. Administered intraperitoneally to mice, chrysin was able to prevent the expression of tonic-clonic seizures induced by PTZ. Ro 15-1788, a central benzodiazepine receptor antagonist, abolished this effect. In addition, all of the treated mice lose the normal righting reflex, which suggests a depressant action of the flavonoid [Medina et al. 1990].

In the elevated plus-maze test, chrysin induced increases in the number of entries into the open arms and in the time spent on the open arms, consistent with an anxiolytic action. In the hole-board assay, chrysin increased the time spent head dipping. In the horizontal wire test, chrysin produced no effects. These data suggest that chrysin possesses anxiolytic actions without inducing sedation and muscle relaxation [Wolfman et al. 1994].

**Matricaria recutita** L. (Chamomile)

Chamomile has been used medicinally for thousands of years and is widely used in Europe. It is a popular treatment for numerous ailments, including sleep disorders, anxiety, digestion/intestinal conditions, skin infections/inflammation (including eczema), wound healing, infantile colic, teething pains, and diaper rash. In the United States, chamomile is best known as an ingredient in herbal tea preparations advertised for mild sedating effects.

German chamomile (*Matricaria recutita*) and Roman chamomile (*Chamaemelum nobile*) are the two major types of chamomile used for health conditions. They are believed to have similar effects on the body, whereas German chamomile may be slightly stronger. Although chamomile is widely used, there is not enough reliable research in humans to support its use for any condition.

The dried flower heads of *Matricaria recutita* L. (Asteraceae) are used in folk medicine to prepare a spasmylytic and sedative tea. The fractionation of the aqueous

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**Figure 18.2** Neuroactive flavones isolated from Pasionaria and chamomile.
extract of this plant led to the detection of several fractions with significant affinity for the central benzodiazepine receptor and to the isolation and identification of 5,7,4′-trihydroxyflavone (apigenin) (Figure 18.2) in one of them. Apigenin competitively binds to the benzodiazepine binding site of the GABA<sub>λ</sub> receptor, and it has no effect on muscarinic receptors, α1 adrenoceptors, or on the binding of muscimol to GABA<sub>λ</sub> receptors. Apigenin has a clear anxiolytic activity in mice in the elevated plus maze without evidencing sedation or muscle relaxant effects at doses similar to those used for classical benzodiazepines, and no anticonvulsant action was detected. Electrophysiological studies performed on cultured cerebellar granule cells showed that apigenin reduced GABA-activated Cl<sup>−</sup> currents in a dose-dependent manner. The effect was blocked by coapplication of Ro 15-1788, a specific benzodiazepine receptor antagonist [Viola et al. 1995; Avallone et al. 2000].

Other studies have also explored the behavioral effects of apigenin and chrysin in rats. These studies demonstrate that the two flavonoids were equally able to reduce locomotor activity when injected in rats. However, whereas chrysin exhibited a clear anxiolytic effect when injected at the dose of 1 mg/kg, apigenin has failed to exert this activity [Avallone et al. 2000; Zanoli et al. 2000].

**Tilia Species (Linden)**

Linden is the common name for the Tiliaceae, a family of chiefly woody shrubs and trees. Most genera are tropical, but the genus *Tilia*, or lime tree, in Europe and Asia, and basswood, in North America, is found throughout the North Temperate Zone. These deciduous trees are valued for ornament and shade. Their light, strong lumber, often called basswood, or whitewood, is variously used (e.g., for woodenware and cheap furniture and for beehives and honeycomb frames).

The dried flowers of these plants have been used widely in herbal teas, as a diuretic, stomachic, antineuralgic, sedative, and tranquilizer around the world. Despite the widespread use of the tea of linden in folk medicine, the number of scientific studies for the evaluation of its therapeutic use is limited.

In an attempt to add experimental confirmation to its popular medicinal use, a pharmacological profile of the chronic administration of the infusion of *Tilia petiolaris* DC., a deciduous tree native to South-East Europe and Western Asia, was performed.

The CNS-related effects of the infusion of *Tilia petiolaris* DC. inflorescences were evaluated in the hole-board, locomotor activity, and light-dark tests in mice. The results suggest that this infusion exerts an anxiolytic-like activity in mice [Loscalzo et al. 2008a]. The explanation of the activities noted must involve a knowledge of which compounds are present in the plant extract. Many studies have reported the CNS activity of *Tilia* extracts, but the isolation and identification of their bioactive principles is scarce. Some studies documented that aqueous extracts of linden produced sedative effects in mice [Coleta et al. 2001]. Viola et al. [1994] described the isolation of a pharmacologically active benzodiazepine binding site ligand from a fraction of the ethanolic extract of *Tilia tomentosa* and the anxiolytic effect exerted by a flavonoid fraction in mice. Recently, it was reported that the hexane, the methanol, and the aqueous extracts of *Tilia americana* var. *mexicana* demonstrated anxiolytic and sedative effects in mice [Aguirre-Hernandez et al. 2007a,b; Herrera-Ruiz et al. 2008; Pérez-Ortega et al. 2008].
To identify the compounds responsible for the tranquilizing effects, pharmacological assay guided purification of a *Tilia petiolaris* DC. inflorescences ethanolic extract was performed. These studies resulted in the isolation and identification of three flavonoid glycosides: isoquercitrin, quercetin 3-O-glucoside-7-O-rhamnoside, and kaempferol 3-O-glucoside-7-O-rhamnoside (Figure 18.3). The behavioral actions of these compounds were examined in the hole-board, locomotor activity, and thiopental-induced loss of righting reflex tests in mice, showing clear depressant activities [Loscalzo et al. 2008b]. These results demonstrate the occurrence of neuroactive flavonoid glycosides in *Tilia*.

![Figure 18.3](image_url) Glycosilated flavonoids isolated from *Tilia*. 

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**Salvia Species (Sage)**

*Salvia* is a genus of plants in the mint family, Lamiaceae. It is one of three genera commonly referred to as sage. This genus includes approximately 700–900 species of shrubs, herbaceous perennials, and annuals with almost worldwide redistribution; the center of diversity and origin appears to be Central and South Western Asia.

The name *Salvia* derives from the Latin “salvere,” which means “to heal.” Indeed, this herb is highly regarded for its healing qualities.

Several types of *Salvia* are used medicinally, such as aromatic varieties (usually strongly scented leaves, also used as herbs), non-aromatic varieties (not considered medicinal, but many still have a scent), Chia sages, and Divinorum (Diviner’s sage) that contains a diterpenoid used for spiritual and recreational purposes and Alzheimer’s disease (research has shown that it improves cognitive function over a period of several months) [Grundmann et al. 2007]. The aromatic sages strengthen the lungs and can be used in teas or tinctures to prevent coughs.

*Salvia* species, such as *S. officinalis* L., *S. lavandulaefolia* Vahl., and *S. miltiorrhiza* Bung. are prominent for their reputed beneficial effects on memory disorders, depression, and cerebral ischemia [Perry et al. 2003].

*S. elegans* Vahl (Lamiaceae), popularly known as “mirto,” is a shrub that has been used widely in Mexican traditional medicine for the treatment of different CNS diseases, principally anxiety. The antidepressant and anxiolytic-like effects of hydroalcoholic (60%) extract of *S. elegans* (leaves and flowers) were demonstrated in mice [Herrera-Ruiz et al. 2006].

**Salvia guaranitica** St. Hil.

*S. guaranitica* St. Hil., is also sometimes called Blue anise sage, anise-scented sage, Brazilian sage, giant blue sage, sapphire sage, or various other common names. It is a species of sage native to South America, including Brazil, Paraguay, Uruguay, and Argentina. It is a popular ornamental plant in mild areas where its leaves purportedly were used by the Guarani Indians of Brazil as a sedative.

Cirsiliol (5,3′, 4′-trihydroxy, 6, 7-dimethoxyflavone) (Figure 18.4) was isolated and identified by a bioguided purification of the ethanolic extract of the aerial parts of *Salvia guaranitica*.

![Figure 18.4 Sedative flavone isolated from Salvia.](image-url)
of *S. guaranitica* St. Hil. Cirsiliol is a competitive low-affinity ligand for the benzodiazepine binding site of the GABA<sub>A</sub> and exerted sedative and hypnotic effects in mice without inducing anxiolysis, muscle relaxation, and prevention of seizures [Marder et al. 1996; Viola et al. 1997].

*Aloysia polystachya* (Griseb.) Moldenke (“Burrito”)

The family Verbenaceae comprises about 175 genus and 2,300 species, distributed in the tropics and subtropics, mainly in the temperate zone of Southern Hemisphere.

*Aloysia polystachya* (Griseb.) Moldenke (Verbenaceae), popularly named “burrito” in Paraguayan folk medicine, is a well-known medicinal plant that has been used for a wide variety of indications, including digestive and respiratory tract disorders. Leaves of *A. polystachya* are used, in Argentina, for respiratory diseases (colds and cough), GI pain, as antiemetic and sedative remedy, or to treat “nervous diseases.” Therapeutic actions of other species of *Aloysia* (i.e., *A. triphilla*) include febrifuge, sedative, stomachic, diuretic, and antispasmodic activities. However, no scientific references or experimental evaluation regarding its CNS activity or toxicity was found.

The study to analyze the behavioral effects of the crude hydroethanolic extract of the aerial parts of *A. polystachya* showed that it exhibits low toxicity, no lethality, did not induce any significant changes in several behavioral and physiological parameters, showed a slight decrease in spontaneous locomotor activity, and showed an increase in breath frequency. The extracts of this plant also evidenced anxiolytic and antidepressant effects in rodents, and these activities are mediated by another mechanism than the benzodiazepine binding site modulation of the GABA<sub>A</sub> receptor [Hellió-Ibarrola et al. 2005, 2008].

**Valeriana Species**

The underground organs of members of the genus *Valeriana* (Valerianaceae) as well as related genera such as *Nardostachys* are used in the traditional medicine of many cultures as mild sedatives and tranquillizers and to aid in the induction of sleep. *V. officinalis, V. wallichii, V. edulis,* and *V. fauriei* are the species most commonly used. It is remarkable that all these species are used for much the same purposes. This plant is still the subject of considerable research aimed at establishing the chemical and pharmacological basis of the activity that has been shown clearly in a number of animal and clinical studies [Houghton 1999].

Valerian is a good example of both the negative and positive aspects of herbal drugs. The considerable variation in its composition and content, as well as the instability of some of its constituents, cause serious problems for standardization, but the range of components that contribute to its overall activity suggest that it may correct a variety of underlying causes of conditions that necessitate a general sedative or tranquilizing effect.

Valerian preparations contained in commercially available products are extracted with water, water/methanol, or water/ethanol mixtures. In several placebo-controlled
clinical studies, an improvement of sleep-related parameters after treatment with aqueous or ethanolic extracts was demonstrated.

In the search for the active substances of *Valeriana*, many compounds have been isolated and identified during the past 120 years, but it is as yet uncertain as to which of them are responsible for the recorded actions [Bos et al. 1996; Houghton 1999].

The most popular compounds, in this connection, are the valepotriates, the baldrinals, and valerenic acid derivatives, as well as some other members of the essential oil.

The presence of a volatile oil in *Valeriana* has been known for a long time, although its characteristic odor, which many find very unpleasant, is attributable to the release of isovaleric acid from some volatile oil components and other constituents by enzyme activity rather than to the oil itself. The composition of the volatile oil is very variable and depends on climate and other ecological factors. The oil contains monoterpenes, chiefly consisting of borneol and its acetyl and isovaleryl esters, but the sesquiterpene components are distinctive and have received most attention regarding their biological activity. Three major types of sesquiterpene skeleton are found, and these are exemplified by valerenic acid, valeranone, and kessyl glycol. The valerenic acid and kessyl ring systems are unique to the Valerianaceae. Valerenic acid has so far been found in no other organism apart from *V. officinalis*, whereas valeranone is found as the major component of the oil of *V. wallichii* and the related plant *N. jatamansi*. Compounds with the kessyl ring system are the major constituents in the volatile oil of Japanese valerian *V. fauriei* but are also found in *V. officinalis* oil [Houghton 1999].

There is certain evidence that valerenic acid may contribute to central effects of extracts derived from *V. officinalis*. It was shown to possess anticonvulsant properties [Hiller and Zetler 1996]. In animal experiments, valerenic acid showed tranquillizing and/or sedative activity [Bent et al. 2006]. This compound also modulates or, at high concentrations, activates GABA_\_A receptors as shown for recombinant receptors expressed in *Xenopus* oocytes [Khom et al. 2007] or neonatal brainstem neurons [Yuan et al. 2004], and its specific binding site on GABA_\_A receptors was identified [Benke et al. 2009].

The valepotriates consist of the furanopyranoid monoterpene skeleton commonly found in the glycosylated forms known as iridoids. They decompose rapidly to give homobaldrlinal and related products. Valepotriates were also discussed to be determinant for central actions of valerian [Andreatini et al. 2002]. They are detectable at trace levels or not at all in recent drug preparations [European Scientific Cooperative on Phytotherapy 2003; Schulz and Hänsel 2004]. The presence of an epoxide group and its alkylating potential, in many of the valepotriates, has raised concerns about their cytotoxicity and consequent potential carcinogenicity.

An approach to detect new active substances in *Valeriana* extracts consists in searching for the presence of ligands for the principal brain receptors predominantly associated with anxiolytic, sedative, and/or sleep-enhancing properties [Marder and Paladini 2002]. Bodesheim and Hözl [1997] found that the lignan (+) hydroxypinoresinol, present in *Valeriana* extracts, is a medium-
low-affinity ligand for the serotonin receptor, but its \textit{in vivo} effects were not investigated.

In addition, the adenosine system may also account for the central action of valerian extracts because, in some studies, an interaction of the extracts, as well as of an olivil derivate at the adenosine A1 receptor, was observed [Müller et al. 2002; Schumacher et al. 2002].

However, several facts have cast doubts on the relevance of the described compounds to explain \textit{Valeriana} extract effects. The principal of them are as follows: (1) the central depressant action of valepotriates, valeranone, and of the essential oil of \textit{Valeriana} could not be demonstrated by a reduction of the glucose turnover in rat brain [Hölzl 1997]; (2) the sedative potency of these compounds is rather low (>30 mg/kg, in mice) [Hölzl 1997]; (3) the valepotriates rapidly decompose, and the baldrinals are chemically reactive and may form polymers [Bos et al. 1996]; hence both valepotriates and baldrinals disappear rapidly from the extracts; and (4) the roots and rhizomes of different \textit{Valeriana} species show large differences with regard to their constituents.

\textit{Valeriana wallichii} DC. and \textit{Valeriana officinalis} L.

In our laboratory, we have applied the “ligand-searching approach” using, as far as possible, purified extracts, and we were able to report the presence of 6-methylapigenin (Figure 18.5) in \textit{V. wallichii} and \textit{V. officinalis} and to prove that it is a benzodiazepine binding site ligand. We have also made the first report of the presence of 2 S(−)-hesperidin (Figure 18.5) in \textit{V. wallichii} and in \textit{V. officinalis} and of linarin (Figure 18.5) in \textit{V. officinalis} and found that they have sedative and sleep-enhancing properties in mice. 6-Methylapigenin, in turn, had anxiolytic activity [Wasowski et al. 2002; Marder et al. 2003, 2005; Fernández et al. 2004]. The effective doses of these compounds are commensurable with their concentrations in the plant extracts and with the doses used in folkloric medicine.

Although isolating and identifying individual chemical constituents with relevant bioactivity provides a rational scientific basis for the medicinal use of a plant, synergistic effects in crude extracts are common. Synergistic interactions are of vital importance in phytomedicines and explain the difficulty in isolating a single active ingredient or to explain the efficacy of low concentrations of active constituents in an herbal product. This concept, that a whole or partially purified extract of a plant offers advantages over a single isolated ingredient, also underpins the philosophy in herbal medicine [Williamson 2001].

CONCLUSION

We have demonstrated that 6-methylapigenin was able to potentiate the sleep-enhancing properties of hesperidin together with a potentiation of the sedative effects by simultaneous administration of linarin with valerenic acid. It was also demonstrated that the potentiated effect of hesperidin is shared with various GABA\textsubscript{A}...
Valeriana wallichii
6-Methylapigenin

V. wallichii and V. officinalis
Hesperidin

Valeriana officinalis
Linarin

Figure 18.5 Neuroactive compounds isolated from Valeriana.

receptor ligands, among them various benzodiazepines widely used in human therapy (alprazolam, bromazepam, midazolam, and flunitrazepam) and with the classical agonist diazepam, in which a synergistic interaction was proved using an isobolar analysis. All the reported data up to now strongly suggest that the behavioral effects induced by hesperidin do not involve classical GABA$_A$ receptors, at least not directly [Fernández et al. 2005, 2006; Loscalzo et al. 2008].

We explored the participation of various other brain receptors besides GABA$_A$, namely opioid, serotonin (5-hydroxytryptamine type 2, 5-HT$_2$) and $\alpha_1$ adrenoceptors, on hesperidin-sedative actions. The endogenous opioid system is critical for many physiological and behavioral effects. They play a major role in pain-controlling systems and also modulate affective behaviors. The serotonergic system is known to modulate mood, sleep, and appetite and, thus, is implicated in the control of numerous behavioral and physiological functions. The $\alpha_1$ adrenoceptors are involved in locomotion, cognitive functions, and the control of motor activity.
The results obtained provide the first pharmacological evidence about the involvement of opioid receptors in the sedative and antinociceptive effects of hesperidin. Our results suggest a possible beneficial use of the association of hesperidin with benzodiazepines not only to improve human sedative therapy but also in the management of pain.

REFERENCES


