Recent Studies on the Zoopharmacognosy, Pharmacology and Neurotoxicology of Sesquiterpene Lactones

Mario Robles¹, Manuel Aregullín², Jan West¹, and Eloy Rodríguez³

¹ Phytochemistry and Toxicology Laboratory, Developmental and Cell Biology, School of Biological Sciences, University of California, Irvine, CA 92717, U.S.A.
² J. H. Bailey Hortorium, Division of Biological Sciences, Cornell University, Ithaca, New York 14853-2703, U.S.A.
³ Address for correspondence

Received: September 19, 1994; Accepted: January 21, 1995

Abstract

Aspects of recent research on the biological activities of sesquiterpene lactones (S(QL)s) are presented. Several S(QL)s have been identified as important constituents of plants consumed by animals for presumed medicinal value and is a focus of research in zoopharmacognosy. Recent in vivo antitumor studies with parthenolide and eupatoriolipin are discussed as well as the reports of the antitumor activity of dehydroleucodin. Helenalin has recently been reported to have cardiotonic activity. Research on the neurotoxicity of repin, a compound reported to cause a Parkinson’s-like disease in horses, is also highlighted.

Key words

Sesquiterpene lactones, zoopharmacognosy, neurotoxins, antitumor, antitumor, cardiotonic, eupatoriolipin, dehydroleucodin, parthenin, repin.

Introduction

Sesquiterpene lactones constitute a large and diverse group of biologically active plant constituents that have been reported from the Acanthaceae, Anacardiaceae, Apiaceae, Euphorbiaceae, Lauraceae, Magnoliaceae, Menispermacae, Rutaceae, Winteraceae, and the Hepatidae (liverworts). However, the greatest number has been reported from the Asteraceae with over 3,000 reported structures. Some of the important medicinal plants such as Arnica montana, Artemisia annua, and Tanacetum parthenium contain sesquiterpene lactones as the active constituents (Fig. 1). Previous major reviews by Rodriguez et al. (1), Fischer (2), Seaman (3), and Picman (4) have discussed the chemistry and taxonomic significance of sesquiterpene lactones; Stupnner and Rodriguez (5) and Picman (4) have reviewed recent aspects of the biological activity. In this short review, we present recent findings in zoopharmacognosy, especially the consumption by chimpanzees of plants containing sesquiterpene lactones for curative purposes (6). We also highlight recent reports and the research on antitumor, antitumor, and cardiotonic activities of sesquiterpene lactones. Lastly, we present our findings on the toxicities of repin, a sesquiterpene lactone reported to be a neurotoxicant, and the subject of extensive toxicological studies (7).

Fig. 1 Sesquiterpene lactones present in medicinal plants of the Asteraceae.

Zoopharmacognosy

The search for novel pharmacological products has further prompted interest in ethnobotanical

* Plenary Lecture presented at the 41st Annual Congress, Society for Medicinal Plant Research, Düsseldorf, Germany, September 1993.
studies of the tropical rainforest regions. Our laboratory has also searched for novel natural products by observing the behavior of primates and other animals that have co-existed and co-evolved with plants of the tropical rainforest for millions of years (8).

Pharmacognosy is the scientific study of the interaction of chemicals being investigated as potential drugs with the biological system of organisms that consume the drug, and requires knowledge of chemistry, isolation techniques, and a familiarity with anatomy and physiology of the animal taking the drug. We have used the term “zoopharmacognosy” to describe the process by which wild animals select and use specific plants with large quantities of natural products for the treatment and prevention of the disease.

The deliberate treatment of diseases with medicines has commonly been assumed to be a unique human trait. However, anecdotal evidence from many naturalists who have observed animals in undisturbed habitats has suggested that animals often do consume plants containing high levels of chemicals that could have medicinal significance. Consequently, we have established collaborative arrangements with naturalists and anthropologists working in several tropical regions of the world to identify novel medicinal compounds.

The most extensive work on zoopharmacognosy of mammals has centered in two areas: plant consumption by chimpanzees and fur rubbing by primates and carnivores. The initial interest of our laboratory derived from the observation that chimpanzees swallowed leaves of *Aspilia mossambicensis* whole without chewing and that this behavior was associated with an apparent alleviation of gastrointestinal distress (9). Bioassay-guided fractionation in our laboratory revealed the presence of a red-colored polyacetylenic compound, thiarubrine A, with an unsaturated dih ring. Subsequent research established that thiarubrine A is a potent antibiotic and has been the object of pharmacological research in our laboratory and the laboratories of Pfizer (10). The investigations of the primatological groups at Harvard and Kyoto have identified a number of plants consumed by chimpanzees for medicinal properties (11, 12). Many of these plants contain significant quantities of sesquiterpene lactones with potent biological activity. Most notably, the anthropologist Michael Huffman (6) working in Mahale Tanzania, observed an ill chimpanzee feeding on *Vernonia amygdalina* for presumed curative properties. Subsequent investigations by Jisaka et al. (13–17) on the chemistry of *V. amygdalina* revealed the presence of the previously reported sesquiterpene lactones vernoldalin, vernoldiol, hydroxyvernoldiol, and vernoldal; and a series of bitter steroidal glycosides, the vernoniosides, of which four have been isolated (Fig. 2). These constituents have been tested for antiparasitic activity by Jisaka and Huffman (15, 17), and they suggested that the steroidal glycosides are more important for their anthelmintic significance than the highly toxic sesquiterpene lactones. However, these investigators assert that sesquiterpene lactones could be crucial for treating the chimpanzee schistosomiasis (13, 14).

![Fig. 2](image)

Sesquiterpene lactones reported to be used by mammals as medicinals.

White-nosed coatis (*Nasua narica*, Procyonidae) of Barro Colorado Island in Panama have been observed by Gompper and Holyman (18), to rub their fur with exuded resin from the trunks of *Trattininickia aspera* (Burseraceae). A chemical study of the resin in our laboratory revealed the presence of the triterpenes α- and β-amyrin, the eudesmane derivative β-selinene, and the sesquiterpene lactone 8β-hydroxyasterolide (Fig. 2). We hypothesize that the lactone may be toxic or repellent to ectoparasites such as fleas, lice, and ticks, as well as biting insects such as mosquitoes.

**Antitumor Activity**

Sesquiterpene lactones have been shown to have both cytotoxic and antitumor activity (19). To date, very little published data has been presented on the *in vivo* antitumor properties of sesquiterpene lactones. In 1982, Towers et al. reported parthenin, derived from *Parthenium hysterophorus*, to have a significant effect on the percentage survivorship of mice given either L1210 or P815 tumor cells (20). The doses Towers used were not lethal to the DBA/2 mice (Fig. 3).

Hladon and Chodera (21) revealed that eupatoriopicrin can prolong the life expectancy of mice after transplantation of the tumors Sa180, EAT, and L1210 (21). In 1987, Woerdenbag et al. reported that eupatoriopicrin possesses strong antitumor activity in the Lewis lung tumor system in mice, an experimental solid tumor system derived from a very malignant type and poorly differentiated epidermoid human carcinoma (22). Woerdenbag et al. concluded that eupatoriopicrin had a cytostatic effect against the solid tumor growth when injected *i.p.* to C57Bl/6 mice. More investigations on the *in vivo* antitumor properties by sesquiterpene lactones are needed.
In 1992, Giordano et al. revised the proposed mode of action and speculated that the protective effect was mediated by more than one mechanism (26). In a similar fashion, Al(OH)$_3$ exerts its protective effect by both stimulating prostaglandins release and forming adducts with the thiol constituents in the gastric mucosa. These thiol constituents are also capable of reacting with the lactones. Structure-activity studies with eighteen sesquiterpene lactones led to the conclusion that the presence of a nonsterically hindered Michael addition acceptor is a requirement for the cytoprotective activity.

Cardiotonic Activity

Some sesquiterpene lactones possess structural similarity to other terpenic natural products. The sesquiterpene lactone helenanin and the grayanotoxins from *Rhododendron* species both contain a hydropyridazine skeleton (27). The grayanotoxins have been shown to exhibit a strong positive ionotropic effect in the guinea-pig heart. This structural resemblance led to the study of the possible cardiotonic activity of helenanin (28).

Strips from the left atrium and papillary muscles from the left ventricle of the guinea-pig heart were used to examine the effect of helenanin on their contractility. Helenanin was shown to produce a positive ionotropic effect (PIE) on both. This positive ionotropic effect is dose-dependent, with concentrations effective on the atrial strips in the range between $10^{-5}$ and $3 \times 10^{-4}$ M, and on the papillary in the range between $3 \times 10^{-4}$ and $10^{-3}$ M. Furthermore, this positive ionotropic effect is irreversible since the PIE does not return to control after washing the preparations. Catecholamine PIE dependency in atrial but not in ventricular muscles was shown in re-inprimized guinea-pigs.

Studies to investigate the mode of action of helenanin concluded that helenanin increases cAMP by inhibiting phosphodiesterase (29). This increase of cAMP causes Ca$^{2+}$ influx enhancing the contractility of the myocardium.

Neurotoxicity

Several plant toxins have been associated with neurodegenerative changes in both brain and spinal cord. Ingestion of a constituent of *Cycas circinalis*, β-N-oxaloylglutamic acid, causes severe neurological disorders in the basal ganglia, cerebral cortex, and spinal cord in man and monkeys, and has been implicated in a neurodegenerative syndrome (30). Until recently, no sesquiterpene lactones have been directly associated with neurological disorders.

However, in 1954, a specific neurological disease of horses commonly known as "chewing disease", was experimentally linked to the ingestion of large amounts of *Centauraea repens* (31). This disorder, which has occurred in California and the southwestern states, is characterized by a lack of facial musculature mobility, idle chewing and tongue flicking, impaired eating and drinking, followed by hypothermia, and lack of reactivity which persists until death. Neuropathological examination of the brain.
from the intoxicated horse revealed bilateral necrosis of the anterior globus pallidus and zona reticulata of the substantia nigra. Cordy (30) termed this disease equine nigro-pallidal encephalomalacia (ENe). The SQGs present in the genus *Centauraea* are concentrated in the aerial parts of the plant as is common in the family Asteraceae (1). To pursue the hypothesis that SQGs might produce neurotoxic effects, an *in vitro* neurotoxicity study was conducted by Stevens and Riopelle (32). This bioassay utilized dorsal root ganglia sensory neurons from an eight-day chick embryo. The growth of the neurites was noted in the presence of the different sesquiterpene lactones from *Centauraea repens* and the 50% toxic dose (TD50) was estimated. On a molar basis, repin was 3–4 times more toxic than its C-17 isomer subluteolide and 4–10 times more toxic than acroptilin and the other SQGs tested (Fig. 3).

In 1991, Hostettmann et al. (33) studied the neurotoxic effects of sesquiterpene lactones from yellow star thistle (*Centauraea solstitialis*). Hostettmann et al. reported that solstitilin and cyanaropicrin exhibited toxicity to cultured rat fetal brain cells in a concentration-dependent manner and suggested that these compounds could cause neurodegenerative disorders. The *in vitro* cell culture of brain tissue is a good neurotoxicity model and has been used widely in the past, notably in the demonstration of the toxicity of MPTP (34, 35).

An important research objective of our laboratory is the study of the biochemical mechanism of action of SQGs. We have suggested that cytotoxicity is mediated by the interaction of the active moiety with enzymes bearing a sulthydryl group (1). This interaction inhibits enzyme activities and metabolism, and interactions with glutathione (GSH) (36).

At present, the etiology of Parkinson’s disease (PD) is not known. The leading hypothesis of PD focuses on the oxidative stress damage to the brain neurons. Researchers have observed a marked decrease in reduced glutathione in the substantia nigra of patients suffering from PD (37). GSH in the substantia nigra and enzymes that utilize GSH for free radical detoxification, such as GSH peroxidase, catalase, and GSH-S-transferase play an important role in protecting dopaminergic nigrostriatal neurons from damage by MPTP. Reduced levels of GSH have been found in the brainstem of mice following administration of MPTP (38, 39). Neurons of the substantia nigra appear to be more vulnerable to oxidative stress because the oxidation metabolism of dopamine has the potential to generate cytotoxic free radicals.

Repin has demonstrated cytotoxicity to different cell lines, and specifically to brain cells. We have also shown repin can deplete levels of intracellular GSH both *in vitro* and *in vivo* in mice (Fig. 4). Depletion of GSH reduces the protection from oxidative stress. Thus, we propose repin toxicity is mediated by the depletion of intracellular GSH.

**Anti-Migraines**

*Tanacetum parthenium*, commonly known as “feverfew”, is widely consumed in England and other regions of northern Europe as a remedy for arthritis and migraine headaches. Several reviews of *T. parthenium* have been published recently, where the botany, phytochemistry, and pharmacology are highlighted (40–43). Heptinstall et al. (44), indicated parthenolide to be the active constituent in feverfew. Parthenolide has been shown *in vitro* to inhibit the synthesis of prostaglandin synthetase, the mediator of inflammation, thereby relieving inflammatory conditions such as arthritis (45). Clinical evidence has demonstrated the efficacy of feverfew for 17 migraine patients (46). Other clinical studies have proven parthenolide to reduce the incidence and severity of migraines (46, 47). Due to the curative properties of feverfew in clinical settings, in the past 10 years parthenolide biological properties have been extensively researched to explain prophylactic activities toward migraines. Parthenolide can inhibit platelet aggregation and serotonin secretion *in vitro* when present at micromolar concentrations (46). The decrease in serotonin levels in platelets is the most consistent of the biological alterations observed in migraines (46). This feature is common to other drugs effective against migraines. Commercial preparations of feverfew are available through pharmacies and health food stores in England. No serious side-effects were reported in the clinical studies, but we know from the literature that sesquiterpene lactones can cause contact dermatitis.

**Conclusions**

The sesquiterpene lactones are an extremely large and diverse group of natural products with an array of biochemical targets that have just begun to be identified. Our laboratory’s research interest in these bio-

---

*Fig. 4* Depletion of glutathione levels *in vitro* and *in vivo* by repin.
logical activities has had a long history and has included work on many organisms, such as bacteria, insects, and vertebrates. A recent focus on the biological activity of compounds from plants consumed by wild animals for presumed medicinal value has once again confirmed the biological significance of the sesquiterpene lactones. Biomedical researchers continue to study these properties and have established that sesquiterpene lactones have potential utility in the treatment of solid tumors, stomach ulcers, and as cardiotonics. Research in our laboratory has also investigated the possibility that certain SQIs are neurotoxic. One SQI, repin, has been implicated in causing a syndrome similar to Parkinson’s disease in horses. Research on the neurotoxicology of sesquiterpene lactones and other novel biological properties will continue to be a focus of numerous laboratories.

Acknowledgements

This research was supported by the National Institutes of Health grants AI18398 and AI24779.

References