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

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Abstract

Aspects of recent research on the biological activities of sesquiterpene lactones (SQLs) are presented. Several SQLs 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 parthenin and eupatoriopicrin are discussed as well as the reports of the antiulcer 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, antiulcer, antitumor, cardiotonic, eupatoriopierin, 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, Menispermaceae, 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; Stuppner 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, antiulcer, 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).

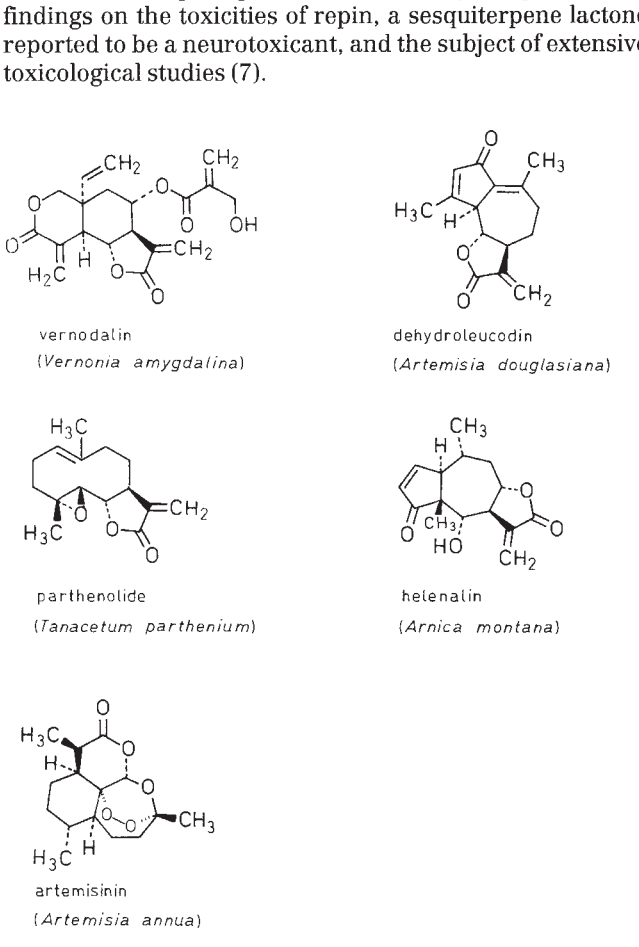

Fig. 1 Sesquiterpene lactones present in medicinal plants of the Asteraceae.

Zoopharmacognosy

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

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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 dithiin 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 primatology 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 feeeding 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 vernodalin, vernolide, hydroxyvernolide, and vernodalol; 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).

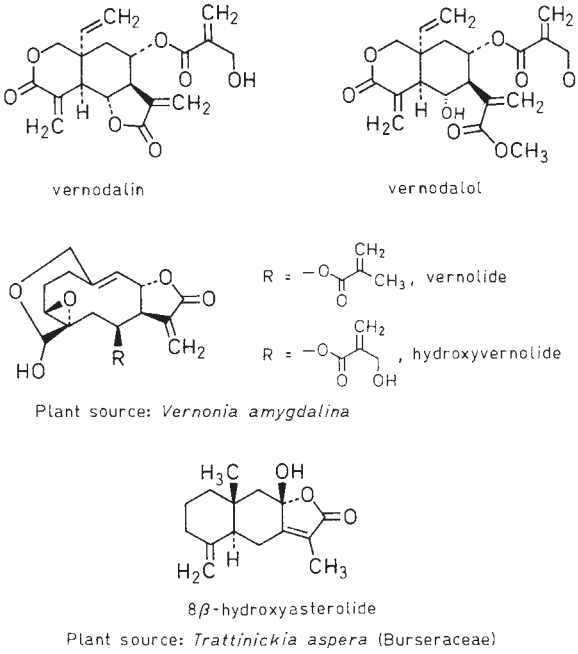

Fig. 2 Sesquiterpene lactones reported to be used by mammals as medicinals.

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 *Trattinnickia 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 C57Bi/6j mice. More investigations on the *in vivo* antitumor properties by sesquiterpene lactones are needed.

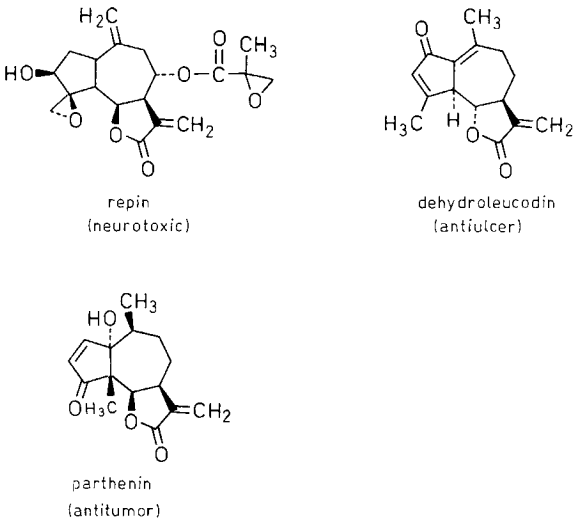

Fig. 3 Sesquiterpene lactones with novel biological activities.

Antiulcer Activity

Plants containing sesquiterpene lactones have been used as herbal remedies in different cultures. In Argentina, leaves of *Artemisia douglasiana* have been used to prepare an infusion called "matico" that is used to treat peptic ulcers, and external sores and ulcers. The *in vivo* gastric cytoprotective properties were investigated by Giordano et al. in 1990 (23). In this study "matico" demonstrated considerable protective effects against the ulcerogenic agents such as absolute alcohol in rats.

The crude chloroformic extract of *A. douglasiana* exhibited significant protective activity and upon chromatographic purification a sesquiterpene lactone, dehydroleucodin, was isolated. Further oral testing demonstrated that this lactone was the antiulcer principle in *A. douglasiana*, and the protective effect was shown to be dose-dependent. It has been speculated by Giordano that the antiulcerogenic activity could be related to the induction of endogenous prostaglandin release. Previously, prostaglandins have been demonstrated to prevent ulcer formation (24, 25). Prostaglandins may protect the surface epithelium against some types of injury (ethanol, aspirin) by increasing the quantity of surface phospholipids which make the mucosal surface hydrophobic and therefore less accessible to acids and other water-soluble agents. When the protective activity of dehydroleucodin was tested in rats pretreated with the prostaglandin release inhibitor, indomethacin, a reduction in the protective effect was observed.

Giordano et al. investigated the activity of other sesquiterpene lactones. The lactones ludartin, 8-angeloyloxy-3-hydroxyguai-3(15),10(4),11(13)-trien-6,12-olide, hymenin, mexicanin I, helenalin, and 9-*O*-deacetylspathulin-2-*O*-angelate, revealed protective activity similar to dehydroleucodin, while the lactone deacetoxymatricarin lacked protective effects (Fig. **3**). Giordano et al. reported that the α-methylene-γ-lactone moiety is required for the protective effect, and the cyclopentenone moiety is not involved in the activity.

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 helenalin and the grayanotoxins from *Rhododrendon* species both contain a hydroperazulene 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 helenalin (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 helenalin on their contractility. Helenalin 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 3×10^{-4} M, and on the papillary in the range between 3×10^{-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 reserpinized guinea-pigs.

Studies to investigate the mode of action of helenalin concluded that helenalin 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*-oxalloylamino-L-alanine, 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 *Centaurea 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 hypokenia, 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 SQLs present in the genus *Centaurea* are concentrated in the aerial parts of the plant as is common in the family Asteraceae (1). To pursue the hypothesis that SQLs 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 *Centaurea repens* and the 50% toxic dose (TD_{50}) 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 SQLs tested (Fig. **3**).

In 1991, Hostettmann et al. (33) studied the neurotoxic effects of sesquiterpene lactones from yellow star thistle (*Centaurea solstitialis*). Hostettmann et al. reported that solstitialin and cynaropicrin 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 SQLs. We have suggested that cytotoxicity is mediated by the interaction of the active moiety with enzymes bearing a sulfhydryl 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.

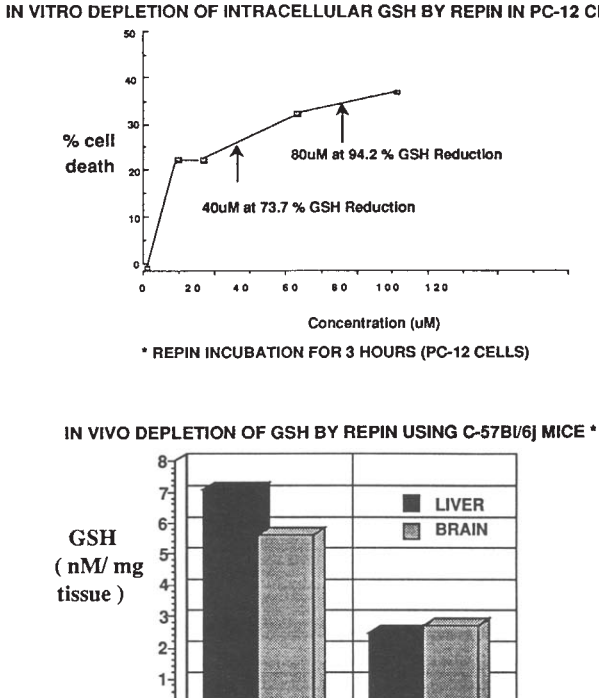

Fig. 4 Depletion of glutathione levels *in vitro* and *in vivo* by repin.

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-

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 SQLs are neurotoxic. One SQL, 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

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References

[1] Rodriguez, E., Towers, G. H. N., Mitchell, J. C. (1976) Phytochemistry 15, 1573–1580.
[2] Fischer, N. H., Olivier, E. J., Fischer, H. D. (1979) The biogenesis and chemistry of sesquiterpene lactones, in: Fortschritt der Chemie organischer Naturstoffe, Vol. 38, (Herz, W., Grisebach, H., Kirby, G. W., eds.), Springer-Verlag, Wien, New York, pp. 47–390.
[3] Seaman, F. C. (1982) Bot. Rev. 48, 121.
[4] Picman, A. K. (1986) Biochem. Syst. Ecol. 14, 255–281.
[5] Stuppner, H., Rodriguez, E. (1987) Phytochem. Bull. 19, 28–38.
[6] Huffman, M. A., Seifu, M. (1989) Primates 30, 51–63.
[7] Robles, M., Rodriguez, E., Yee, S., Choi, B. (1995) Repin, a sesquiterpene lactone with potential neurotoxicity, manuscript in preparation.
[8] Rodriguez, W., Wrangham, R. (1993) in: Phytochemical Potential of Tropical Plants, (Downum, K. R., ed.), Chapter 4, "Zoopharmacognosy: The Use of Medicinal Plants by Animals", Plenum Press, New York.
[9] Rodriguez, E., Aregullin, M., Nishida, T., Uehara, S., Wrangham, R. W., Abramowski, Z., Finlayson, A., Towers, G. H. N. (1985) Experientia 41, 419–420.
[10] Pfizer Pharmaceuticals (1991) unpublished results.
[11] Wrangham, W. R., Goodall, J. (1989) Primates 24, 276–282.
[12] Nishida, T. (1990) In: The Chimpanzees of the Mahale Mountains: Sexual Strategies and Life History, (Nishida, T., ed.), pp. 22–37, University of Tokyo Press, Tokyo.
[13] Jisaka, M., Kawanaka, M., Sugiyama, H., Takegawa, K., Huffman, M. A., Ohigashi, H., Koshimizu (1992) Phytochemistry 56, 845–846.
[14] Jisaka, M., Ohigashi, H., Takegawa, K., Hirota, M., Irie, R., Huffman, M. A., Koshimizu, K. (1993) Phytochemistry 34, 409–413.
[15] Jisaka, M., Ohigashi, H., Takagaki, T., Nozaki, H., Tada, T., Hirota, M., Irie, R., Huffman, M. A., Nishida, T., Kaji, M., Koshimizu, K. (1992) Tetrahedron 48, 625–632.
[16] Jisaka, M., Ohigashi, H., Takegawa, K., Huffman, M. A., Koshimizu, K. (1993) Biosci. Biotech. Biochem. 57, 833–834.
[17] Jisaka, M., Kawanaka, M., Sugiyama, H., Takegawa, K., Huffman, M. A., Ohigashi, H., Koshimizu, K. (1992) Biosci. Biotech. Biochem. 56, 845–846.
[18] Gompper, M. E., Holyman, A. M. (1993) J. Tropical Ecology 9, 533–540.
[19] Kupchan, S. M., Eakin, M. A., Thomas, A. M. (1971) J. Med. Chem. 14, 1147.
[20] Towers, G. H. N., Mew, D., Balza, F., Levy, J. G. (1982) Planta Med. 45, 23–27.
[21] Hladon, B., Chodera, A. (1975) Arch. Immunol. Ther. Exp. 23, 857–865.
[22] Woerdenbag, H. J., Lemstra, J., Hendricks, H., Malingre, T. M., Konings, A. W. T. (1987) Planta Med. 53, 318–322.
[23] Giordano, O. S., Guerreiro, E., Pestchanker M. J. (1990) J. Nat. Prod. 53, 803–809.
[24] Miller, T. (1983) Am. J. Physiol. 243, 601–23.
[25] Tarnawski, A., Hollander, D. (1985) Gastroenterology 89, 366–374.
[26] Giordano, O. S., Pestchanker, M. J., Guerreiro, E., Saad, J. R., Enriz, R. D., Rodríguez, A. M., Jáuregui, E. A., Guzmán, J., María, A. O. M., Wendel, G. H. (1992) J. Med. Chem. 35, 2452–2458.
[27] Itoigawa, M., Takeya, K., Furukawa, H., Ito, K. (1987) J. Cardiovasc. Pharmacol. 9, 193–201.
[28] Takeya, K., Itoigawa, M., Furukawa, H. (1983) Chem. Pharm. Bull. 31, 1719–1725.
[29] Ivie, G. W., Witzel, D. A. (1983) Handbook of Natural Toxins, (Keeler, R. F., Tu, A. T., eds.), Academic Press, New York.
[30] Cordy, D. R. (1954) J. Neuropath. Exp. Neurol. 13, 330–342.
[31] Stevens, K. L., Wong, R. Y. (1990) J. Nat. Prod. 53, 218.
[32] Stevens, K. L., Riopelle, J. (1993) in: *In vitro* Neurotoxicity Bioassay: Neurotoxicity of Sesquiterpene Lactones, (Colegate, S., ed.), Chapter 19, "Bioactive Natural Products, Detection, Isolation, and Structural Determination", CRC Press, Boca Raton, Florida.
[33] Hostettmann, K., Hamburger, M. (1991) Helv. Chim. Acta 74, 117–123.
[34] Barnes, J. M. (1989) Brit. J. Pharm. 96, 332.
[35] Michel, P. (1989) J. Pharm. Expt. Therap. 248, 842–850.
[36] Lee, K., Hall, I. H. (1977) J. Med. Chem. 20, 911–914.
[37] Jenner, P. (1993) J. Neural Transm. 40 ([Suppl.]), 23–36.
[38] Riederer, P. (1986) J. Neurochem. 46, 1359–1365.
[39] Yong, V. W., Krisman, A. A. (1986) Neuroscience 63, 56–60.
[40] Warren, R. G. (1986) Aust. J. Pharm. 67, 475.
[41] Heptinstall, S. (1988) J. R. Soc. Med. 81, 373.
[42] Hobb, C. (1989) Herbal Gram 20, 26.
[43] Awang, D. V. C. (1989) Can. Pharm. J. 122, 266.
[44] Heptinstall, S., White, A., Williamson, L., Mitchell, J. R. A. (1985) Lancet, 1071.
[45] Pugh, W. J., Sambo, K. (1989) J. Pharm. Pharmacol. 40, 743–745.
[46] Johnson, E. S., Kadam, N. P., Hylands, D. M., Hylands, P. J. (1985) Br. Med. J. 291, 569–573.
[47] Murphy, J. J., Heptinstall, S., Mitchell, J. R. A. (1989) Lancet, 189.