General Behavior, Toxicity, and Cytotoxic Activity of Elenoside, a Lignan from *Justicia hyssopifolia*

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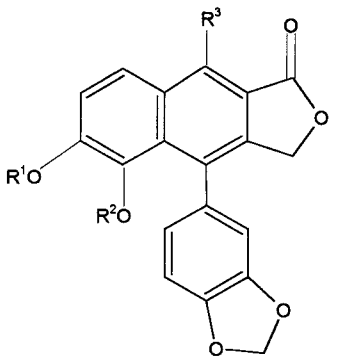
Cytotoxicity, acute toxicity, and general pharmacological effects in mice of an arylnaphthalene lignan isolated from *Justicia hyssopifolia* were studied. Elenoside was cytotoxic to human cancer cell lines in a range of concentrations from 10^{-5} to 10^{-4} M. It has an LD_{50}, ip in mice, of 305 mg/kg and central depressive properties at doses of 25, 50, and 100 mg/kg. Thus, elenoside appears to be a sedative with broad spectrum cytotoxicity.

Introduction

A number of arylnaphthalene lignans have been isolated from different species of *Justicia,* many of which exhibit diverse biological activities, including antitumor,[1,2] antiviral, insecticidal, cardiotonic, analgesic, inhibition of lipid peroxidation, antiinflammatory, platelet activating factor antagonism, and central nervous system depression and stimulation properties. For example, (+)-nortrachelogenin causes depression in rabbits,[3] and prostalidins A, B, and C produce a mild depression in rats and mice.[4] The bis-epoxylignan glycoside simplexoside causes depression in mice and rats, while its aglycone is a stimulant.[4]

We previously reported the isolation of a new arylnaphthalene lignan elenoside (β-D-glucoside) (**2**) and its aglycone (**1**) from *Justicia hyssopifolia* L. (Acanthaceae),[5] which is also the source of hyssopifoline and the known lignans helioxanthin, justicidin E, gadain, and cubebin. We now report the biological effects results of elenoside.



	R^1	R^2	R^3
1	O—CH_2—O		OMe
2	H	Glc	H

The LD_{50} (24 h) of elenoside in mice is 305 ± 7 mg/kg by ip route. No lethality was observed for 5 days following administration of elenoside.

An examination of the general pharmacological effects of elenoside (E) using the Irwin test[6] revealed that elenoside reduced spontaneous activity (E-25 = 3, E-50 = 2.8, and E-100 = 1.3 versus control = 4 at 60 min after administration) as a function of time and dose. There was a decrease in the motor affective response (E-25 = 3, E-50 = 2.8, and E-100 = 1.3 versus control = 4), which, in part, reflects the effect of elenoside on social behavior. The dose-dependent reduction of motor affective and sensory-motor responses (E-25 = 3, E−-50 = 2.8, and E-100 = 2.2 versus control = 4) denotes an action similar to drugs such as major tranquilizers, sedative-hypnotics, and narcotic analgesics.[6] Major changes in neurologic and autonomic areas usually denote untoward side effects.[6] Elenoside produced a decrease in muscular tone (E-25 = 3, E-50 = 2.8, and E-100 = 2.2 versus control = 4), a loss of equilibrium (E-25 = 0.2, E-50 = 0.2, and E-100 = 0.4 versus control = 0), and a hypotonic gait (E-25 = 0.8, E-50 = 1.2, and E-100 = 1.8 versus control = 0). Muscular tone decreased in a dose-dependent manner, and this reduction continued through 180 min. The loss of righting reflex and hypotonic gait, although slight, was also evident 30 min after administration. These results suggest that elenoside is similar in action to the sedative-hypnotic effect of the barbiturates. No CNS excitation (i.e., tremors, twitches, and convulsions) was observed. The presence of palpebral closure, hypothermia, reduction in respiratory rate, and piloerection, as well as the manifestation of diarrhea, indicates some autonomic nervous system involvement. No deaths were observed within 24 h post-treatment or for 5 days thereafter.

When elenoside was compared with a major tranquilizer (chlorpromazine = CHL) for effects on spontaneous movement, muscle tone, and righting reflex, it was found that elenoside administration (25 and 50 mg/kg) resulted in loss of spontaneous movements (CHL = 90%, E-25 = 50% and E-50 = 60% of animals utilized), muscular tone (CHL = 90%, E-25 = 50% and E-50 = 60% of animals utilized), and righting reflex tone (CHL = 40%, E-25 = 20% and E-50 = 20% of animals utilized) at 60 min after administration. Thus, elenoside exhibits CNS-depressant action similar to that of other psychopharmacological agents, although the exact nature and category of such actions cannot be determined from the present data.

Elenoside was cytotoxic to leukemia cell lines (CCRF-CEM, K-526, MOLT-4, RPMI-8226) at a concentration of 10^{-4} M (79−97% growth inhibition). Some activity was also observed (at 10^{-4} M concentration) against specific melanoma cell lines (M19-MEL, 81% growth inhibition; SK-

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MEL-2, 84% growth inhibition), a CNS cancer cell line (SNB-19, 81% inhibition), a renal cancer cell line (UO-31, 80% inhibition), and a colon cancer cell line (HCC-2998, 87% inhibition). At concentrations less than 10^{-4}, elenoside was not significantly cytotoxic to any of the cell lines.

Lignans obtained from *Justicia pectoralis* L., *Sesbaina drummondii* L., and others are known to be cytotoxic to leukemia and solid tumor cell lines.[7] A derivative of podophyllotoxin (VP-16-213 etoposide) shows similar results in a panel of human tumor lines with important effects on small cell lung cancer, leukemia, and other solid tumors.[2] Also, arylnaphthalene lignans obtained from *Hyptis verticillata* L. showed cytotoxicity against a panel of cell lines comprising a number of human cancer cell types (breast, colon, fibrosarcoma, lung, prostate, KB, Kb-VI, and murine lymphocytic leukemia (P-388)).[8]

Similarly, the results show elenoside, which is a lignan structurally related to prostalidins,[4] the justicidins, and the podophyllotoxin derivatives,[9] is also cytotoxic at concentration between 10^{-5} and 10^{-4} M. The findings of the present study indicate that this compound exhibits central depressant activity and cytotoxicity at concentrations of 10^{-5}–10^{-4} M. Therefore, we believe that elenoside has an interesting biological activity profile that should be studied further. Such investigations of elenoside and elenin derivatives as potential anticancer agents are in progress in our laboratory.

Experimental Section

Plant Material. *Justicia hyssopifolia* L. belongs to the Acanthaceae family and is an endemic species in the Canary Islands.[10] Leaves of *J. hyssopifolia* L. were collected in October 1990 at Punta Cangrejo, Adeje, Tenerife. A voucher specimen is deposited at the Herbarium of the Department of Botany, Faculty of Biology, University of La Laguna (TFC-28938).

Extraction and Isolation of Elenoside. Air-dried and ground leaves were extracted in a Soxhlet with EtOH to afford an extract that was concentrated under reduced pressure. Column chromatography using solvents of increasing polarity (*n*-hexane, EtOAc, EtOH) gave different groups of fractions. Elenoside was recovered as needles from the most polar fractions of the ethanolic extract and was identified by comparison of its spectroscopic data (NMR and MS) with previously reported data.[5]

Animals. Swiss albino male mice (weighing 25−30 g) were used. The animals were housed under normal laboratory conditions at 22 °C on a standard light−dark schedule (12:12; lights on: 0800 to 2000) and were given free access to standard laboratory diet and water. The animals were assigned to randomized groups of 10 each. The elenoside was suspended in a mixture of propyleneglycol−ethanol−plant oil−Tween 80 (40:10:50:2) and administered intraperitoneally. The control group received the vehicle.

Acute Toxicity. A total of 50 male mice (25−30 g) were allotted to the different control and test groups. The control group received the vehicle, and test groups received doses of 167, 209, 262, 327, and 512 mg/kg of elenoside. The animals were kept in plastic cages (10 animals per cage) and observed for 5 days. The LD_{50} of elenoside was estimated using the Spearman−Kärber method.[11]

General Behavior: Irwin Test. Forty male mice (25−30 g) were assigned to four randomized groups of 10 each. Groups were observed at 30, 60, 120, and 180 min following the ip administration of vehicle and 25 (E-25), 50 (E-50), and 100 mg/kg (E−-100) of elenoside. Changes in behavior and neurological, autonomic, and toxic reactions were noted, as described by Irwin.[6]

Lim Test. Measurements of animal behavior were determined as described by Lim,[12] with slight modifications[13] (different number of animals and times for evaluating behavior changes). This test was performed using 40 male mice (25−30 g) that were assigned to four randomized groups of 10 each. Groups were observed before and after administration of vehicle, 25 and 50 mg/kg of elenoside, or chlorpromazine, 10 mg/kg, during 3 h. Mice were evaluated for spontaneous movements, muscular tone, and righting reflex.[12]

Statistical Analyses. Statistical analyses were performed using the CSS program and analysis of variance (ANOVA) followed by the Student's *t*-test or chi-square test. Differences were considered significant when associated with a probability of 5% or less ($p \leq 0.05$).

In Vitro Cytotoxicity Studies. The cytotoxicity profile of elenoside (NSC 644013-W/1) was studied in a human tumor cell line panel, which constitutes the disease-oriented, in vitro drug-screening system of the National Cancer Institute (NCI). Detailed descriptions of the standard technical procedures and data performed by the NCI have been already published.[14]

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Supporting Information Available: The data of the Irwin test, Lim test, and in vitro cytotoxicity. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Moritani, S.; Nomura, M.; Takeda, Y.; Miyamoto, K. *Biol. Pharm. Bull.* **1996**, *19*, 1515−1517.
(2) O'Dwyer, P. J.; Leyland-Jones, B.; Alonso, M. T.; Marsoni, S.; Wittes, R. E. *N. Engl. J. Med.* **1985**, *312*, 692−700.
(3) Kato, A.; Hashimoto, Y.; Kidohoro, M. *J. Nat. Prod.* **1979**, *42*, 159−162.
(4) Ghosal, S.; Banerjee, S.; Jaiswal, D. *Phytochemistry* **1980**, *19*, 332−334.
(5) Trujillo, J.; Jorge, E.; Navarro, E.; Boada. J. *Phytochemistry* **1990**, *29*, 2991−2993.
(6) Irwin, S. *Psychopharmacologia* **1968**, *13*, 222−257.
(7) Hui, Y.; Chang, C.; McLaughlin, J. *J. Nat. Prod.* **1986**, *49*, 1175−1176.
(8) Novelo, M.; Cruz, J. G.; Hernández, L.; Pereda-Miranda, R.; Chai, H.; Mar, W.; Pezuto. J. M. *J. Nat. Prod.* **1993**, *56*, 1728−1736.
(9) Dewick, P. M.; Jackson, E. *Phytochemistry* **1981**, *9*, 2277−2280.
(10) Bramwell, D.; Bramwell, Z. *Flores Silvestres de Las Islas Canarias*; Rueda: Madrid, 1990; p 276.
(11) *International Encyclopedia of Pharmacology and Therapeutics (Section 7), Biostatistics in Pharmacology*; Delanois, A. L., Ed.; Pergamon Press: Oxford, 1973; Vol. II, pp 984−986.
(12) Lim, R. K. S. In *Pharmacologic Techniques in Drug Evaluation*; Nodine, J. H., Siegler, P. E., Eds.; Year Book Medical Publishers Inc.: Chicago, 1964; p 294.
(13) Watanabe, K.; Goto, Y.; Yoshitomi, K. *Chem. Pharm. Bull.* **1973**, *21*, 1700−1708.
(14) Boyd, M. R. *Princ. Pract. Oncol. Updates* **1989**, *3*, 1−12.

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