

# Cytotoxicity of Sesquiterpene Lactones<sup>1</sup>

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## SUMMARY

A number of naturally occurring sesquiterpene lactones have been studied for cytotoxic activity against three different cell lines. The results obtained show that the most immediate and direct factor responsible for cytotoxicity of the compounds studied is the introduction of the  $O=C-C=CH_2$  system.

## INTRODUCTION

The recent discovery and structure elucidation of approximately 100 new sesquiterpene lactones during a systematic study of *Artemisia* and other genera of the family of *Compositae* in our laboratory (16) have provided the opportunity for further investigations on the potential of these sesquiterpene lactones as new therapeutic agents. Much of the earlier study on sesquiterpene lactones for medicinal purposes has concentrated mainly on santonin (I), a santanolide, and its derivatives, which are well known as important anthelmintic and ascaricidal agents (21). With the increased activity in recent years in searching for tumor inhibitors or cytotoxic principles from a random screening of plant sources, a program supported in the United States and in many other parts of the world by the Cancer Chemotherapy National Service Center, National Cancer Institute, NIH, many sesquiterpene lactones bearing an  $\alpha$ -methylene- $\gamma$ -lactone grouping have occasionally been isolated and shown to contain significant antitumor or cytotoxic activity. These include the germacranolides, elephantin, elephantopin (see Ref. 26 and the references therein), costunolide, and tulipinolide (10); the guaianolides, gaillardin (see Ref. 27 and the references therein), euparotin acetate, and its companions (see Ref. 26 and the references therein), the pseudoguaianolide, damsine (11); and the elemanolide, vernolepin (see Ref. 26 and the references therein).

Although the structural requirements for the antitumor or cytotoxic activity of some of the above sesquiterpene lactones have been postulated (see Ref. 31 and the references therein), and attempts have been made to explain their mechanism of action (see Refs. 26 and 31 and the references therein), there has been a need for further study of other structural types of the sesquiterpene lactones, such as santanolides and

xanthanolides, the antitumor or cytotoxic activities of which have not yet been reported. We have selected for investigation a variety of structural types (Charts 1 to 5) for cytotoxicity screening, since this type of preliminary screening can be used as an invaluable guide for selecting possible antitumor agents. This communication correlates the structure-activity relationship based upon the results obtained from our preliminary cytotoxicity screening with 3 different cell lines according to a rapid microtiter method which will be reported in a separate paper.<sup>2</sup>

## MATERIALS AND METHODS

Unless otherwise specified, the naturally occurring sesquiterpene lactones used in this study are all analytical samples and were obtained from the studies previously reported as shown in Table 1. The selection of these compounds was based mainly upon the availability of the samples as well as their characteristic structural types.

Helenalin (XVI) was isolated from the extraction of *Helenium microcephalum* M. A. Curt. ex Gray according to an exact procedure described in the literature (28). Helenalin, m.p. 170–172° (benzene) [literature (3), m.p. 169–172° (benzene)] and its acetate, helenalin acetate (XVII),<sup>3</sup> m.p. 180–180.5° (dichloromethane-ether) [literature (3), m.p., 179.5–180.5° (aqueous methanol) (3)], prepared from acetylation of helenalin with pyridine-acetic anhydride in the usual manner, were identified by their comparable melting points as well as the spectral (UV, infrared, nuclear magnetic resonance) data as reported by Clark (see Ref. 8 and the references therein), Adams, Herz *et al.* (3–5, 12, 22), Büchi and Rosenthal (6), and others.

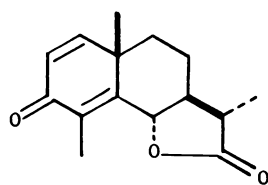
The cytotoxicity tests were performed in a microtest plate in which different concentrations of sample compounds were simultaneously tested against 3 different cell lines which originated from normal human fibroblasts, human laryngeal carcinoma, and human cells transformed with simian virus 40. These cell lines were grown in Eagle's minimal essential medium (Grand Island Biochemicals) with 10% fetal bovine serum and 100 units/ml of neomycin. The stock cells were fed fresh growth (with 10% serum) 24 hr before use in the test. The cell suspension was well dispersed by gentle pipetting in the proper amount of growth medium and then diluted to a final concentration of about  $10^5$  cells/ml. The sesquiterpene

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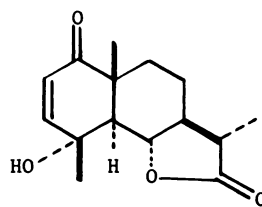
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<sup>2</sup>E. S. Huang, K. H. Lee, C. Piantadosi, T. A. Geissman, J. S. Pagano, A Rapid Microtiter Method for Cytotoxicity Screening, paper in preparation.

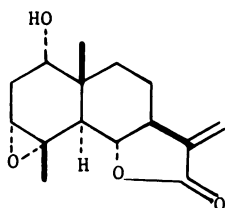
<sup>3</sup>K. H. Lee, R. Meck, and C. Piantadosi, unpublished results.



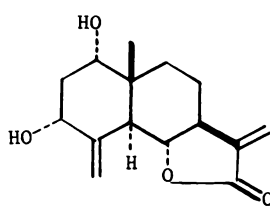
$\alpha$ -santonin (I)



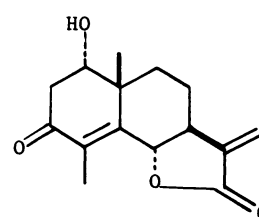
vulgarin (II)



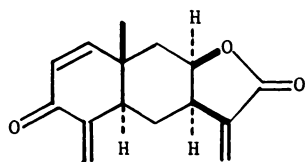
ludovicin-A (III)



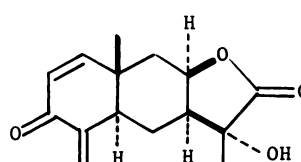
ludovicin-B (IV)



ludovicin-C (V)

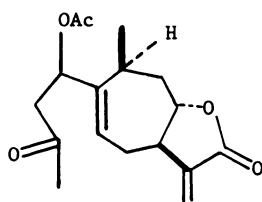


encelin (VI)

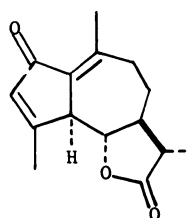


farinosin (VII)

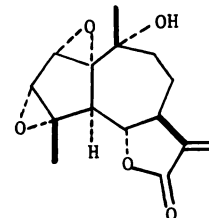
Chart 1. Santanolides.



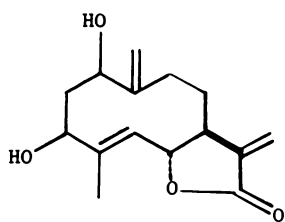
xanthinin (VIII)  
Chart 2. Xanthanolide.



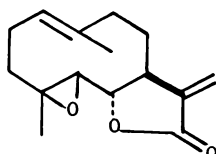
deacetoxymatricarin (XI)



canin (XII)

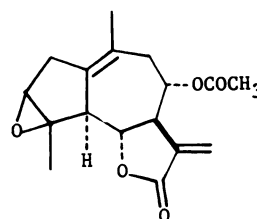


ridentin (IX)

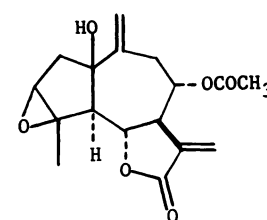


parthenolide (X)

Chart 3. Germacronolides.



arteglaasin-A (XIII)

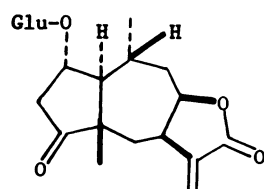


arteglaasin-B (XIV)

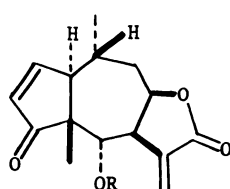
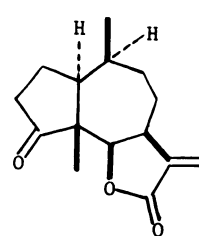
Chart 4. Guaianolides.

lactones were dissolved in dimethyl sulfoxide at a concentration of 10 mg/ml and diluted with the growth medium to the desired drug concentration, for example, 50  $\mu$ g/0.9 ml, 10  $\mu$ g/0.9 ml, 4  $\mu$ g/0.9 ml, etc. Microtest plates with 60 wells (Falcon No. 3034) were used for the assays. Each well with a flat bottom holds about 0.02 ml of medium. The mixtures of 0.1 ml of cell suspension ( $1 \times 10^5$  cell/ml)

and 0.9 ml of diluted sample were inoculated into the culture well with a tuberculin syringe fitted with a 22-gauge needle. One drop of the mixture ( $\sim 0.02$  ml) was placed in the testing well by gently rotating the barrel of the syringe. All culture plates were incubated in a water-jacketed  $\text{CO}_2$  incubator with 5%  $\text{CO}_2$  at 37° without changes of the media. All assays were examined daily for 1 week with a low-power inverted



paucinin (XV)

R=H, helenalin (XVI)  
R=COCH<sub>3</sub>, helenalin acetate (XVII)

damsine (XVIII)

Chart 5. Pseudoguaianolides.

Table 1  
Cytotoxicity of sesquiterpene lactones

No.	Compounds	Reference	ED <sub>50</sub> (μg/ml) <sup>a</sup>		
			WI-38 <sup>b</sup>	H.Ep. 2 <sup>c</sup>	W-18 Va2 <sup>d</sup>
I	α-Santonin <sup>e</sup>		>100	>100	>100
II	Vulgarin	15	19.23	51.55	16.04
III	Ludovicin A	28	5.70	6.32	3.89
IV	Ludovicin B	28	7.36	7.52	5.68
V	Ludovicin C	28	1.99	5.30	1.77
VI	Encelin	18	0.25	0.92	0.56
VII	Farinosin	18, 23	0.23	0.60	0.74
VIII	Xanthinin	9, 13, 14, 37	0.10	0.62	0.14
IX	Ridentin	24	1.33	3.05	1.58
X	Parthenolide	17 and references therein	0.18	0.76	0.32
XI	Deacetoxymatricarin	19	>20	>20	>20
XII	Canin <sup>f</sup>	30	0.99	3.07	3.00
XIII	Arteglasin A <sup>f</sup>	29	0.17	1.91	0.72
XIV	Arteglasin B <sup>f</sup>	29	0.32	1.89	0.63
XV	Paucinin	35	0.04	0.19	0.08
XVI	Helenalin <sup>g</sup>	3-6, 8, 12, 22,	0.03	0.18	0.07
XVII	Helenalin acetate	3-5, 8	0.08	0.29	0.07
XVIII	Damsin <sup>h</sup>	1, 2, 11	0.21	2.33	2.15

<sup>a</sup> The values of ED<sub>50</sub> were determined based upon the rapid microtiter method described.<sup>2</sup>

<sup>b</sup> WI-38 refers to normal human diploid fibroblasts.

<sup>c</sup> H.Ep. 2 refers to human epidermoid carcinoma of larynx.

<sup>d</sup> W-18 Va2 refers to simian virus 40-transformed cells of human origin.

<sup>e</sup> Marketed by K & K Laboratories, Inc., Plainview, N.Y.

<sup>f</sup> These 3 epoxy-containing guaianolides were shown to exhibit a gradual decrease in their potency of cytotoxic activity as the testing time was prolonged.

<sup>g</sup> The cytotoxicity and antitumor activity of helenalin were first determined by workers at Research Triangle Institute, M. E. Wall, M. C. Wani, and H. Taylor (paper submitted to *Lloydia*).

<sup>h</sup> Purified by recrystallization from dichloromethane-ether. The value of ED<sub>50</sub> of this compound obtained by the Cancer Chemotherapy National Service Center according to the procedure described (33) is 0.32 μg/ml (11). The apparent differences in values of ED<sub>50</sub> as shown is attributed by using different methods and cell lines which have been discussed previously.<sup>2</sup>

binocular microscope (100X, Olympus). Between 2 and 7 days after inoculation, the percentage of the area of the bottom surface occupied by adhering surviving cells can be roughly estimated. By the day in which the control cells reached 100% confluence, the average percentage of confluence area of 6 assay cultures was designated as the degree of assay cell growth. The ED<sub>50</sub><sup>4</sup> is used for expressing the potency of cytotoxicity. The above cytotoxicity test procedures will be described in detail elsewhere.<sup>2</sup>

Damsin (XVIII), m.p. 108–109°, the major constituent of *Ambrosia maritima* L. (1, 2) the cytotoxicity of which has

<sup>4</sup>The abbreviation used is: ED<sub>50</sub>, calculated effective dose that inhibits the net cell growth to 50% of control growth.

been assayed under the auspices of the Cancer Chemotherapy National Service Center screening program (11), has been used as a reference standard sample.

## RESULTS AND DISCUSSION

The ED<sub>50</sub> values for the cytotoxicity obtained from the evaluation of 18 sesquiterpene lactones with 3 different cell lines are summarized in Table 1. In addition, the plots of degree of cell growth as compared with control growth versus drug concentration of 6 selected compounds are shown in Chart 6. The results clearly suggest the following conclusions.

The most basic structural requirement for the cytotoxic

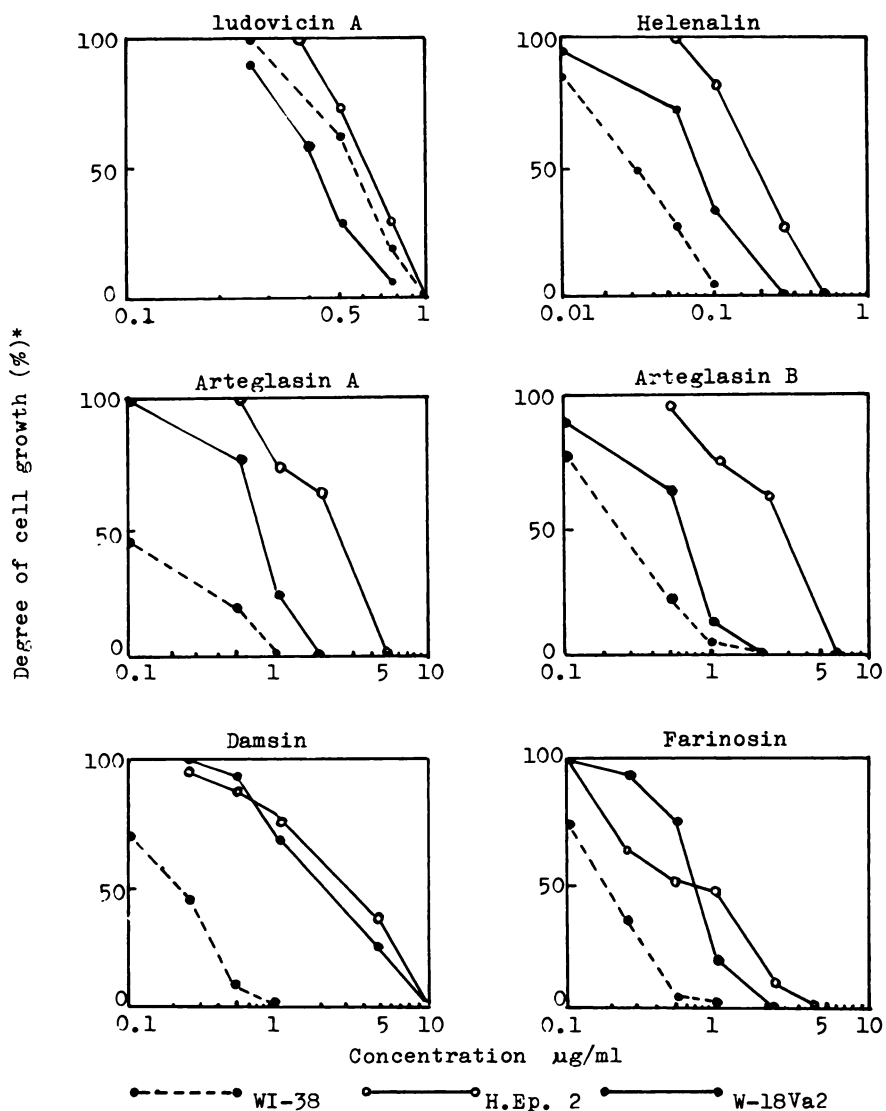


Chart 6. Plots showing the growth inhibition as a function of drug concentration. Asterisk on abscissa, see literature.

activity of the above sesquiterpene lactones is mainly due to the introduction of an  $\alpha$ -methylene- $\gamma$ -lactone moiety in the molecule regardless of the difference in structural types. Thus, hydrogenation of the conjugated  $\alpha$ -methylene- $\gamma$ -lactone systems, as in  $\alpha$ -santonin (I), vulgarin (II), and deacetoxyatricarin (XI), leads to essentially inactive compounds.

That the  $\alpha$ -methylene- $\gamma$ -lactone grouping plays an important role as enzyme-alkylating agent is well known (Refs. 7, 25, 26, and 31; see also references cited in Refs. 26 and 31). Furthermore, a recent study on the mechanism of action of the inhibition of phosphofructokinase has been demonstrated to involve this moiety (20).

Comparison of the cytotoxic activity of encelin (VI) and farinosin (VII) disclosed that they are about equally active, suggesting that the principal active center is probably the  $\alpha, \beta$ -unsaturated ketone, in which the methylene grouping is exocyclic, since this can act in the same way as the  $\alpha$ -methylene- $\gamma$ -lactone. This hypothesis shows that the

necessity is not for the unsaturated lactone but for the  $O=C-C=CH_2$  system whether it be in the lactone or ketone. An example of this type would be sarkomycin (XIX), *i.e.*, 2-methylene-3-oxocyclopentanecarboxylic acid, a simple antibacterial and antitumor antibiotic (see Ref. 32 and the references therein). Studies on the significance of the  $\alpha, \beta$ -unsaturated ketone for antitumor or cytotoxic activity are currently underway.



The angular santanolides, ludovicin A (III), B (IV), and C (V), are the less cytotoxic ones of the different structural classes examined, although they possess actual or potential

bifunctional alkylating groups, such as an epoxide in ludovicin A, an  $\alpha$ -methylene- $\beta$ -hydroxyl in ludovicin B, and an  $\alpha,\beta$ -unsaturated ketone (but not exocyclic methylene) in ludovicin C, in addition to the  $\alpha$ -methylene- $\gamma$ -lactone moieties. (For effective alkylating agents, it is usually necessary for them to contain 2 functional groups as described in Refs. 34 and 36, although some monofunctional alkylating agents also possess antitumor activity.) However, unlike the other structures (VI to X and XII to XVIII except XVII), which show a higher ED<sub>50</sub> for WI-38 than that for W-18 Va2, these types of compounds revealed some selectivity against tumor virus-transformed cell growth; for example, the ED<sub>50</sub> of ludovicin A is smaller (more cytotoxic) for W-18 Va2 (3.89) than that for WI-38 (5.70). Further extensive study on this class of compounds, which possess potential selectivity against growth of virus-transformed cells, is currently in progress.

The compound with highest cytotoxic activity found in Table 1 is helenalin (XVI) (ED<sub>50</sub> for H.Ep. 2, 0.18), a linear pseudoguaianolide containing, in addition to the  $\alpha$ -methylene- $\gamma$ -lactone moiety, an  $\alpha,\beta$ -unsaturated ketone system, i.e., 2 alkylating functions in the molecule; consequently, its high level of cytotoxic activity may be due to the inhibition of DNA synthesis by interstrand cross-linking of the DNA twin helix, as postulated by others (Refs. 31, 34, and 36; see also references cited in Ref. 31). The exact mechanism of action of helenalin and related compounds is currently under investigation and will be reported.

Other potent cytotoxic structures with at least 2 alkylating groups are found in the bicyclic xanthanolide, xanthinin (VII) and the germacranolide, parthenolide (X). That the highly cytotoxic monoalkylating compound, paucin (XV), a glucosidic pseudoguaianolide, is about 20 times more cytotoxic than damsine (XVIII) clearly suggests that a carrier moiety such as glucose can have a profound influence on the cytotoxic activity by assisting in the transport processes.

Further investigations on the structure-activity relationships between the antitumor or cytotoxic activity and the structures of sesquiterpene lactones are in progress.

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