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Inhibition of DNA Biosynthesis in HeLa Cells by Cytotoxic and Antitumor Sesquiterpene Lactones

JAN M. WOYNAROWSKI AND JERZY KONOPA

Technical University of Gdansk, Department of Pharmaceutical Technology and Biochemistry, Gdansk, Poland

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SUMMARY

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Parthenolide, a cytotoxic sesquiterpene lactone, was found to inhibit incorporation of radioactive thymidine, uridine, and leucine into macromolecules in HeLa cells. In the case of the two latter precursors, the inhibitory effects were predominantly due to decreased uptake of uridine and leucine into cells, which indicated a negligible effect of parthenolide on RNA and protein synthesis. In contrast, inhibition of thymidine incorporation into DNA was only partially caused by impaired uptake of the precursor into cells. This result was directly demonstrated in experiments with HeLa cells pulse-labeled with [^{3}H]thymidine at 6° and chased at 37° in the presence of parthenolide. The inhibition of thymidine incorporation into DNA, as observed under these conditions, seems to reflect the interference of parthenolide with DNA synthesis, since the metabolic conversion of thymidine to deoxythymidine triphosphate remained unaffected. Moreover, incubation of HeLa cells with parthenolide resulted in the inhibition of [^{3}H]deoxythymidine triphosphate incorporation into DNA in nuclear systems ("lysates") derived from these cells. Hence, the inhibitory action of parthenolide probably occurs at the replication level as opposed to the effect on the biosynthesis of DNA precursors. Several other cytotoxic and antitumor sesquiterpene lactones were also found to inhibit DNA synthesis in HeLa cells. This inhibitory activity appeared to correlate with the cytotoxic activities of sesquiterpene lactones against HeLa cells in the case of eight compounds among nine studied. The results suggest that DNA is a target molecule in the mechanism of action of cytotoxic and antitumor sesquiterpene lactones.

INTRODUCTION

Sesquiterpene lactones constitute one of the largest classes of cytotoxic and antitumor substances of plant origin (1-4). Despite the wide interest in these compounds, their mode of antitumor action is known only to a small extent. The principle requirement for biological activity of sesquiterpene lactones is the presence of an exocyclic methylene conjugated to an α-lactone or β-substituted conjugated cyclopentenone ring (1, 5-8). The presence of an additional functional group as an α,β-unsaturated side chain ester, an α,β-unsaturated ketone, or chlorohydrin enhances the cytotoxicity of sesquiterpene lactones and is generally required for antitumor activity *in vivo* (1, 5, 9). These groupings can act as the alkylating centers in the Michael-type reaction of sesquiterpene lactones with nucleophiles, such as sulfhydryl or amino groups (7, 8, 10-13). Some antitumor sesquiterpene lactones have been found to inhibit phosphofructokinase and glycogen synthetase reactions *in vitro*, coincidentally with loss of sulfhydryl groups from the enzymes (13, 14). On the basis of these observations, Hanson *et al.* (13) and Smith *et al.* (14) postulated that selective alkylation of sulfhydryl groups in biologically important enzymes is the major mechanism by which sesquiterpene lactones exert their effects on cell growth. However, another possibility for the mode of action of these substances, namely the alkylation of DNA, should also be considered. First, despite the fact that reaction with DNA was not observed *in vitro* (8), it may occur in the cell via metabolic activation of sesquiterpene lactones. Second, alkylation of DNA, even if it occurs to a limited extent, may lead to an important biological consequence (for review see ref. 15). In contrast, a much higher degree of alkylation of protein seems to be necessary to block the functions of enzymes present in cells in numerous copies. Third, the mutagenic properties of

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a sesquiterpene lactone hymenovin (16, 17) suggest a direct interference with DNA function. Fourth, assuming that alkylation of proteins is the most important event, it is difficult to explain why additional alkylating groups greatly enhance cytotoxic activity or are even necessary for the activity *in vivo* of sesquiterpene lactones. This observation could easily be explained by the ability of the multifunctional alkylating compounds to form DNA interstrand cross-links and/or DNA-protein cross-links. Fifth, the hypothesis of Kupchan *et al.* (5) is based almost entirely on observations in a simple-model cell-free system, although these investigators found a lack of correlation between the cytotoxic activity of sesquiterpene lactones and their ability to alkylate sulfhydryl groups.

Direct studies of binding to DNA in cells would require labeled sesquiterpene lactones of relatively high specific radioactivity. Since such compounds are difficult to obtain, we attempted to ascertain whether cytotoxic and antitumor sesquiterpene lactones exert biochemical effects which could result from alkylation of DNA.

In this study, we demonstrated that parthenolide, a cytotoxic sesquiterpene lactone, preferentially inhibits DNA biosynthesis in HeLa cells, most probably by acting directly on the replication process. Moreover, we found that several other cytotoxic and antitumor sesquiterpene lactones are also able to inhibit DNA biosynthesis in HeLa cells, and this effect seems to play a role in the cytotoxic action of the compounds studied.

MATERIALS AND METHODS

Sesquiterpene lactones. Parthenolide was isolated from *Tanacetum vulgare* L by chromatography of the chloroform extract on a silica gel column, and 1,10-epoxy parthenolide was obtained by epoxidation of parthenolide with *m*-chlorperbenzoic acid[1]. Other sesquiterpene lactones were generously provided by Professor B. Drozdz, the Medical Academy, Poznan, Poland (alatholide, eupatoriopicrine, and grossheimin); Dr. A. T. Sneden, the University of Virginia, Charlottesville, Va. (vernolepin and elephantopin); and Professor R. W. Doskotch, Ohio State University, Columbus, Ohio (costunolide and epitulipinolide).

Solutions of sesquiterpene lactones for cytotoxic activity determinations were prepared in ethanol. In other experiments, lactones were dissolved in dimethyl sulfoxide. The final concentration of ethanol or dimethyl sulfoxide in the media was 0.25% or 0.1%, respectively.

Tissue culture. HeLa cells were grown in a monolayer culture in Eagle's minimal essential medium supplemented with 5% fetal calf serum and antibiotics. All experiments involving radioactive thymidine, uridine, or leucine were carried out using a cell suspension prepared in the following way: HeLa cells were washed with PBS,[2] trypsinized, washed with PBS, and resuspended in cold (4°) Eagle's minimal essential medium for suspension culture (Flow Laboratories, Irvine, Scotland) with 0.01% methylcellulose and without serum and antibiotics. When the uptake and incorporation of [^{14}C]leucine were assayed, the medium without leucine was used.

Total cellular radioactivity and incorporation of radioactive precursors into macromolecules in HeLa cells incubated with radioactive precursors at 37° (System A). Aliquots of cell suspension (0.35×10^6 cells/ml) were given sesquiterpene lactone as indicated and radioactive precursor, [^{3}H]thymidine (7 Ci/mmole), [^{3}H]uridine (24.4 Ci/mole), or D,L-[^{14}C]leucine (20.4 mCi/mmole), to a final concentration of 2 μCi/ml. The cells were incubated at 37° for an indicated time, after which 1-ml samples were withdrawn. Samples for total cellular radioactivity (duplicates) were diluted with 6 ml of cold PBS and immediately centrifuged (1800 × *g* for 2 min). The cellular pellets were washed again with PBS, suspended in 0.3 ml of 0.5 M TCA, and hydrolyzed for 45 min at 70°. Radioactivity of the samples was determined in Triton X-100-containing scintillation fluid [3 g of 2,5-diphenyloxazole, 260 ml of Triton X-100 (Sigma Chemical Company, St. Louis, Mo.), 105 ml of ethanol, 35 ml of ethylene glycol, and 600 ml of xylene] in a liquid scintillation counter (Isocap/300, Nuclear Chicago Corporation, Chicago, Ill.). Samples for incorporation of labeled precursors into macromolecules (duplicates) were diluted with 9 ml of ice-cold 10% (w/v) TCA with 1% (w/v) sodium pyrophosphate. After at least 2 hr of standing at 0–4°, the precipitated macromolecules were centrifuged (2000 × *g* for 5 min) and washed three times with 5% (w/v) TCA, 6 ml each time. The samples were then hydrolyzed and radioactivity was counted as described above.

Total cellular radioactivity and incorporation of [^{3}H]thymidine or [^{3}H]uridine into macromolecules in HeLa cells preincubated with radioactive precursors at 6° (System B). This experimental system was based on that developed by Plageman (18). The cell suspension (0.35×10^6 cells/ml) was incubated for 30 min at 6° with either [^{3}H]thymidine or [^{3}H]uridine at a concentration of 4 μCi/ml. The suspension was then centrifuged (600 × *g* for 5 min) at 0°, and the cells were washed with ice-cold PBS and resuspended in fresh medium (0.35×10^6 cells/ml). Sesquiterpene lactones were added and the cells were incubated at 37° for the indicated times. Samples were withdrawn and processed as described above for System A.

Phosphorylation pattern of [^{3}H]thymidine. HeLa cells were preincubated with [^{3}H]thymidine at 6° and washed and incubated for 10 min at 37° as described above (System B), except that the cell density was 0.7×10^6 cells/ml and the [^{3}H]thymidine concentration was 5 μCi/ml. Cold PCA extracts from these cells were obtained and processed according to the method of Plageman (18). Resulting samples were applied to a polyethyleneimine cellulose thin-layer chromatographic plate (Merck and Company, Darmstadt, West Germany) previously developed with water. The chromatogram was developed with 0.2 M LiCl (1.5 cm), then with 0.5 M LiCl (7 cm), and finally with 0.8 M LiCl (9 cm). The following R_F values were obtained in this system: 0.15 for dTTP, 0.32 for dTDP, 0.56 for dTMP, and 0.83 for thymidine. The zones of the cellulose layer were removed and incubated for 2 hr with water (0.1 ml) and 1.2 ml of 1 M hyamine

[1] J. M. Woynarowski and J. Konopa, unpublished data.

[2] The abbreviations used are: PBS, phosphate-buffered saline (17 mM NaCl, 0.27 mM KCl, 8.1 mM Na_2HPO_4, 0.2 mM KH_2PO_4, pH 7.2); TCA, trichloroacetic acid; PCA, perchloric acid; dTTP. dTDP, dTMP, deoxythymide tri-, di-, and monophosphates; dGTP, dUTP, dCTP, deoxyguanosine, deoxyuridine, and deoxycytosine triphosphates.

hydroxide solution in methanol. The radioactivity of the samples was determined in a standard toluene-containing scintillator.

DNA synthesis in HeLa cell lysates. HeLa cells growing in monolayer culture were incubated for 1 hr with parthenolide. The density of cells in the medium was 0.35×10^6/ml. The preparation of cell lysates and assay for DNA synthesis in the lysates were carried out generally according to the method of Fraser and Huberman (19). The cells were washed with Buffer T,[3] trypsinized, suspended in Buffer T, centrifuged ($800 \times g$ for 5 min), washed with Buffer H,[4] and resuspended in Buffer H at a concentration of 2.5×10^7 cells/ml. After 10 min, the cells were homogenized using a Dounce homogenizer. Lysates were combined (7:1) with an assay mixture (19) supplemented with [^{3}H]dTTP. The final concentrations in the incubation mixture were as follows: 36 mM KCl; 9 mM Na_2HPO_4; 45 mM 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid; 8 mM glucose; 2 mM dithiothreitol; 1 mM EDTA, 0.45 mM ethylene glycol bis(β-aminoethyl ether)-*N*,N′-tetraacetic acid, 12 mM $MgCl_2$; 30 mM spermine; 4 mM ATP; 0.7 mM each for GTP, UTP, and CTP; 0.1 mM each for dCTP, dATP, and dGTP; and 0.03 mM dTTP (26 μCi/ml), final pH 7.9. Aliquots (100 μl) of the incubation mixture were incubated at 37° for the indicated time. Incubation was terminated by the addition of 3 ml of cold 0.6 M PCA with 5 mM sodium pyrophosphate. After at least 2 hr of standing in ice, the tubes were centrifuged at 0° ($2000 \times g$ for 8 min) and the pellets were washed four times with cold 0.4 M PCA containing 5 mM sodium pyrophosphate. The samples were hydrolyzed and the radioactivity was measured as described for precursor incorporation into intact cells.

Cytotoxic activity. The cytotoxic activity of sesquiterpene lactones against HeLa cells was determined according to the method of Smith *et al.* (20). Results are expressed as molar concentrations of sesquiterpene lactones inhibiting by 50% the growth of HeLa cells. The determinations were repeated three times, using at least three concentrations of each compound producing growth inhibitions in the range of 10–90%.

RESULTS

Most of the experiments reported in this paper were conducted with parthenolide (Compound I, Fig. 1). The structures of the other sesquiterpene lactones used in these studies are given in Fig. 1.

By using a routine procedure to measure macromolecule biosynthesis in HeLa cells (System A; see Materials and Methods), we found that parthenolide inhibits the incorporation of labeled thymidine, uridine, and leucine into acid-insoluble material (Table 1). Parthenolide exerted the most pronounced effect on thymidine incorporation, inhibiting this process almost completely at a concentration of 202 μM after only a few minutes of incubation with the cells (Table 1; Fig. 2B). A markedly lower concentration of lactone (40 μM) was sufficient to produce over 90% inhibition when cells were incubated with parthenolide for 3 hr (data not shown).

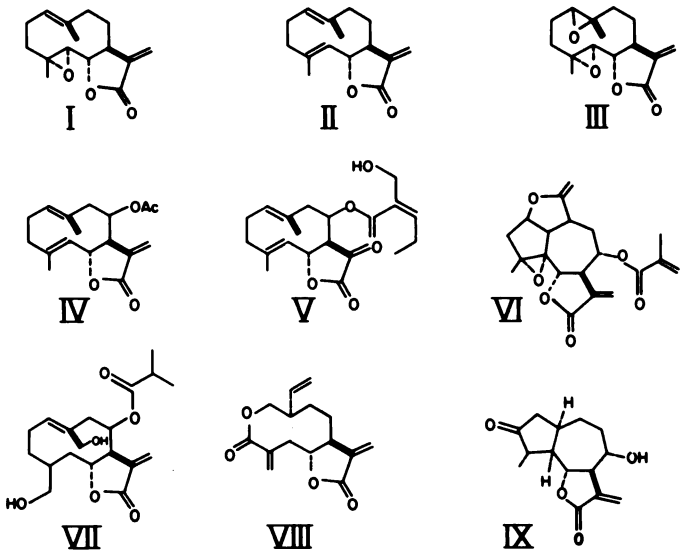

FIG. 1. *Structures of sesquiterpene lactones*
I, Parthenolide; *II,* costunolide; *III,* 1,10-epoxyparthenolide; *IV,* epitulipinolide; *V,* eupatoriopicrin; *VI,* elephantopin; *VII,* alatolide; *VIII,* vernolepin; *IX,* grossheimin.

The decreased incorporation of macromolecule precursors into cells might reflect interference with macromolecule biosynthesis but could also result from other mechanisms, e.g., decreased uptake of radioactive precursors into cells or impaired metabolic conversion to triphos-

TABLE 1

Effect of parthenolide on incorporation of radioactive precursors into macromolecules and on the uptake of these precursors by HeLa cells in System A

The cells were incubated at 37° for 25 min with inhibitor and [^{3}H]-thymidine, [^{3}H]uridine, or [^{14}C]leucine. Results represent average values for duplicate samples from two to four experiments and are expressed as percentages of control ± standard error of the mean.

	Parthenolide concentration	[^{3}H]thymidine	[^{3}H]uridine	[^{14}C]leucine
	μM	%	%	%
Incorporation into macromolecules[a] (%)	0	100 ± 1.5	100 ± 1.5	100 ± 4.3
	41	42.3 ± 0.4		
	81	22.3 ± 1.6	48.3 ± 2.5	42.5 ± 4.4
	202	4.4 ± 0.1	32.7 ± 1.0	22.7 ± 0.6
	404	1.1 ± 0.2		
Uptake into cells[b] (%)	0	100 ± 0.7	100 ± 2.7	100 ± 2.2
	41	51.3 ± 3.5		
	81	40.0 ± 3.2	63.4 ± 4.4	49.9 ± 5.8
	202	17.7 ± 0.6	38.2 ± 2.1	29.1 ± 2.9
	404	9.5 ± 1.3		

[a] Radioactivity values in controls were approximately 1×10^5 dpm, 0.4×10^5 dpm, and 0.5×10^4 dpm for [^{3}H]thymidine, [^{3}H]uridine, and D,L-[^{14}C]leucine, respectively.

[b] Radioactivity values in controls were approximately 2×10^5 dpm, 1.5×10^5 dpm, and 1×10^4 dpm for [^{3}H]thymidine, [^{3}H]uridine, and D,L-[^{14}C]leucine, respectively.

[3] Buffer T (19): 0.137 mM NaCl, 0.005 M KCl, 0.007 M $NaHPO_4$, 0.025 M Tris-HCl, pH 7.8.

[4] Buffer H (19): 2 mM $MgCl_2$, 2 mM dithiothreitol, 1 mM EDTA, 10 mM Na_2HPO_4, pH 8.0.

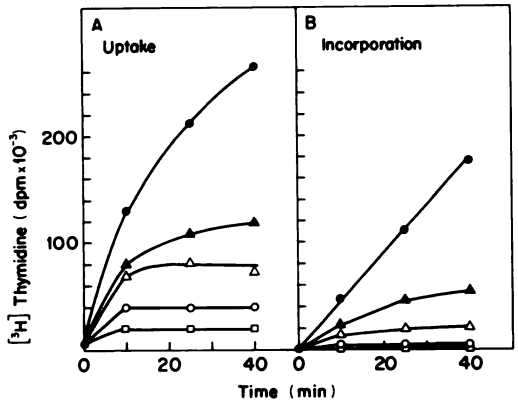

FIG. 2. *Time course of the effect of parthenolide on the uptake of [^{3}H]thymidine into HeLa cells (Panel A) and on the incorporation of [^{3}H]thymidine into macromolecules in HeLa cells (Panel B)*

The cells were incubated at 37° with parthenolide and [^{3}H]thymidine (System A). Parthenolide concentrations: ●, 0 μM (control); ▲, 41 μM; △, 81 μM; ○, 202 μM; □, 404 μM. Each point represents the mean of duplicate samples.

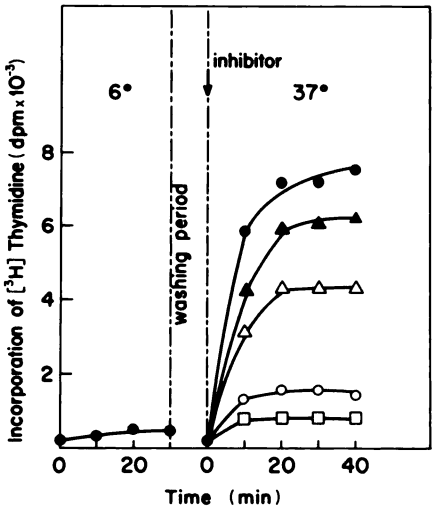

FIG. 3. *Effect of parthenolide on the incorporation of [^{3}H]thymidine into macromolecules in HeLa cells preincubated at 6° with [^{3}H]thymidine and then incubated at 37° in fresh medium with parthenolide without labeled precursor (System B)*

Parthenolide concentrations: ●, 0 μM (control); ▲, 41 μM; △, 81 μM; ○, 202 μM; □, 404 μM. Each point represents the mean of duplicate samples.

phates in the case of thymidine and uridine. To elucidate the former possibility, we assayed the effect of parthenolide on the uptake of radioactive precursors into HeLa cells by measuring the total radioactivity present in the cells incubated with parthenolide and [^{3}H]thymidine, [^{3}H]uridine, or [^{14}C]leucine. Parthenolide appeared to decrease the uptake of each of these precursors into HeLa cells (Table 1). The uptake of uridine or leucine into cells seemed to be inhibited to an extent similar to the incorporation of these precursors into macromolecules (Table 1). This observation suggests that the latter effect was entirely or almost entirely due to an inhibition of uridine and leucine uptake. In contrast, in the case of [^{3}H]thymidine, incorporation into DNA was suppressed by parthenolide more markedly than was the uptake of this precursor into cells (Table 1; cf. Fig. 2A and 2B).

To establish unequivocally whether the effect of parthenolide on thymidine incorporation reflects the inhibition of DNA synthesis and is not due only to inhibition of thymidine uptake, we performed a set of experiments in the following manner (referred to further as System B). HeLa cells were preincubated for 30 min at 6° in medium containing [^{3}H]thymidine. Under these conditions, thymidine was taken up by the cells, but was not incorporated into DNA (Fig. 3, *left*). After preincubation, the cells were quickly washed and incubated at 37° with lactone in a fresh medium without the radioactive precursor. This protocol (System B) enabled us to bypass the inhibition of the precursor transport by measuring the incorporation of the labeled precursor that was present in the cells when the inhibitor was added. Figure 3 and Table 2 show that parthenolide in System B suppressed the incorporation of [^{3}H]thymidine into DNA in a dose-dependent manner, and almost complete inhibition was observed at a lactone concentration of 404 μM. The possibility that the decreased incorporation of [^{3}H]thymidine was due to leakage of radioactivity from parthenolide-treated cells was excluded, since the lactone did not affect significantly the total cellular radioactivity under these conditions (Table 2).

An experimental protocol similar to System B was used to measure the incorporation of [^{3}H]uridine in the cells preloaded with the labeled precursor at 6° and then chased at 37° in the presence of parthenolide. Lactone at a concentration of 81 μM or 202 μM appeared to have only a slight influence on the incorporation of [^{3}H]uridine and total cellular radioactivity in this assay. These results confirmed the interpretation that the inhibition of uridine incorporation in System A (Table 1) was predominantly caused by an impaired uptake of the precursor as opposed to a real effect on RNA synthesis.

The effect of parthenolide on thymidine incorporation in System B might result from the impaired metabolic conversion of thymidine. To exclude this possibility, we assayed the distribution of radioactive label in PCA extracts from HeLa cells preincubated with [^{3}H]thymidine at 6° and then incubated at 37° with or without parthenolide. Chromatography of the control extracts on

TABLE 2

Effect of parthenolide on the incorporation of nucleic acid precursors into macromolecules and total cellular radioactivity in HeLa cells in System B

The cells were preincubated for 30 min at 6° with either [^{3}H]-thymidine or [^{3}H]uridine and then incubated for 20 min at 37° with parthenolide in fresh medium without labeled precursor. Results represent average values for duplicate samples from two to four experiments and are expressed as percentages of control ± standard error of the mean.

Parthenolide concentration	Incorporation into macromolecules[a]	
	[^{3}H]thymidine	[^{3}H]uridine
μM	%	%
0	100 ± 2.0 (100 ± 4.4)	100 ± 0.8 (100 ± 2.0)
41	74.1 ± 4.9 (102 ± 9.3)	
81	61.2 ± 1.8 (92.6 ± 4.7)	95.6 ± 1.7 (97.9 ± 6.9)
202	24.3 ± 1.3 (115.0 ± 1.8)	73.2 ± 2.1 (88.5 ± 1.6)
404	12.5 ± 0.8 (116.6 ± 3.4)	

[a] Values in parentheses indicate total cellular radioactivity.

polyethyleneimine cellulose revealed that 84% of the label corresponded to dTTP, 3% to dTDT, 11% to dTMP, and 2% to thymidine. The values for extracts from cells treated with parthenolide (404 μM, 10 min) were 79% for dTTP, 5% for dTDP, 14% for dTMP, and 2% for thymidine, respectively.

To elucidate further the effect of parthenolide on DNA synthesis, we assayed the incorporation of [^{3}H]dTTP into DNA in a nuclear system ("lysate") derived from parthenolide-treated HeLa cells. A 1-hr incubation of HeLa cells with 202 μM and 404 μM parthenolide resulted in a pronounced reduction of the subsequent incorporation of [^{3}H]dTTP into nuclei (Fig. 4).

The effects of several other cytotoxic and antitumor sesquiterpene lactones (Fig. 1) on DNA synthesis were determined by measuring [^{3}H]thymidine incorporation into DNA in System B. All of the compounds assayed were found to inhibit this process (Fig. 5). Lactone concentrations producing 50% inhibition in this system were estimated graphically from the plots shown in Fig. 5 and were denoted ID_{50} values. These numbers are given in Table 3 together with the cytotoxic activities of the compounds studied, expressed as ED_{50} values (lactone concentration inhibiting by 50% the growth of HeLa cells). Regression analysis of the ID_{50} plot versus the ED_{50} plot by the least-squares method revealed a statistically significant correlation between the two activities (Eq. 1) when Compound IV (epitulipinolide) was excluded from the calculations:

$$ID_{50} = 19.4 \times ED_{50} + 37.4 \qquad (1)$$
$$r = 0.803 \qquad n = 8$$

where r is the correlation coefficient and n is the number of compounds included in the analysis.

DISCUSSION

A cytotoxic sesquiterpene lactone, parthenolide, was found to decrease the uptake of thymidine, uridine, and leucine into HeLa cells and the incorporation of these precursors into macromolecules. It is known that intact sulfhydryl groups are necessary for the function of transport proteins in cellular membranes (21). On the other hand, biologically active sesquiterpene lactones in general react easily with sulfhydryl groups (7, 8, 10–13). Thus, the decreased uptake of macromolecular precursors by parthenolide could be explained by alkylation of the transport proteins. Moreover, it seems very likely that other biologically active sesquiterpene lactones also affect membrane function.

In the case of parthenolide the inhibition of uridine and leucine incorporation into macromolecules is predominantly due to the decreased uptake of these precursors, thus parthenolide apparently has little effect on RNA and protein synthesis. In contrast, the incorporation of thymidine into DNA is also strongly inhibited by parthenolide under conditions where the lactone effect on the precursor uptake is bypassed (System B).

The extent of the parthenolide effect on thymidine

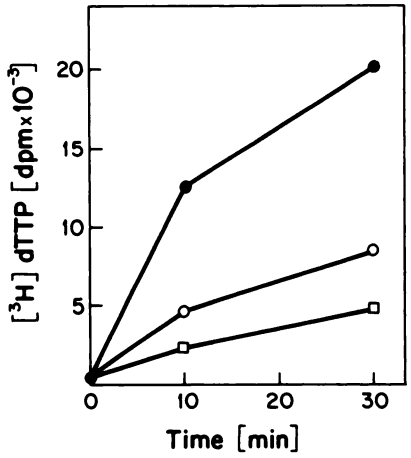

FIG. 4. *Effect of parthenolide on the incorporation of [^{3}H]dTTP into DNA in "lysates" from HeLa cells preincubated for 1 hr with inhibitor*

Parthenolide concentrations: ●, 0 μM (control); ○, 202 μM; □, 404 μM. Each point represents the mean of triplicate samples.

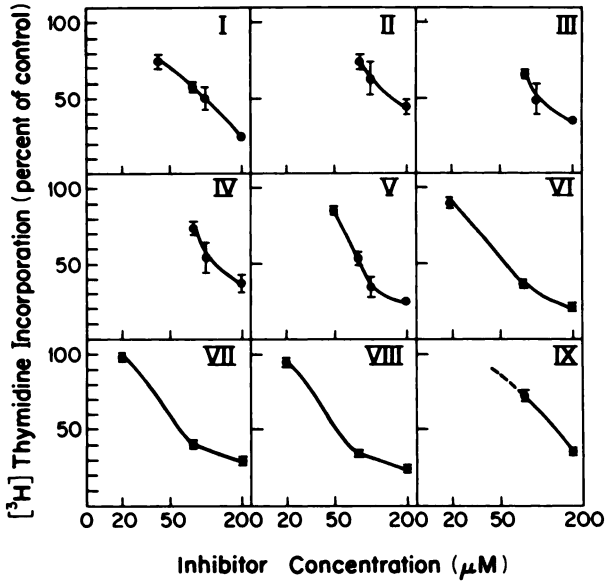

FIG. 5. *Inhibition of [^{3}H]thymidine incorporation into macromolecules by various sesquiterpene lactones in HeLa cells preincubated for 30 min at 6° with [^{3}H]thymidine and then incubated for 20 min at 37° in fresh medium with inhibitor but without labeled precursor (System B)*

I, Parthenolide; *II*, costunolide; *III*, epoxyparthenolide; *IV*, epitulipinolide; *V*, eupatoriopicrin; *VI*, elephantopin; *VII*, alatolide; *VIII*, vernolepin; *IX*, grossheimin.

TABLE 3

Cytotoxic activities of sesquiterpene lactones (ED_{50}) and their ability to inhibit incorporation of [^{3}H]thymidine into macromolecules (ID_{50}) in HeLa cells in System B

	Compound	ED_{50}[a]	ID_{50}[a]
		μM	μM
I	Parthenolide	3.00 (0.14)	99 (12)
II	Costunolide	6.15 (0.43)	163 (7)
III	Epoxyparthenolide	5.35 (0.03)	121 (11)
IV	Epitulipinolide	1.10 (0.07)	129 (12)
V	Eupatoriopicrin	1.15 (0.10)	80 (7)
VI	Elephantopin	0.05 (0.02)	53 (13)
VII	Alatolide	3.10 (0.14)	59 (6)
VIII	Vernolepin	2.40 (0.25)	50 (20)
IX	Grossheimin	3.45 (0.08)	142 (19)

[a] Values in parentheses are ranges.

incorporation observed in System B (i.e., with cells preincubated with [^{3}H]thymidine at 6° and chased at 37° in the presence of the inhibitor) was in very good coincidence with the incorporation in System A (cells incubated at 37° with both parthenolide and [^{3}H] thymidine) when the latter values were corrected for the reduced uptake (data not shown). These data point to the advantages of System B for studying the influence on DNA and RNA biosynthesis in the case of agents which inhibit (or are suspected to inhibit) the uptake of thymidine or uridine into cells.

In the case of parthenolide, which apparently had no effect on the conversion of thymidine to dTTP, the decreased incorporation of [^{3}H]thymidine in System B reflects the inhibition of DNA synthesis. This conclusion is further supported by the observation that incubation of HeLa cells with parthenolide results in a decreased incorporation of [^{3}H]dTTP into DNA in the nuclear system (lysate) derived from these cells. The degree of inhibition of DNA synthesis as measured in lysates was close to that found by incorporation of [^{3}H]thymidine in System B. Moreover, the lysate system provided an excess of all immediate precursors of DNA replication. Thus, the effect of parthenolide on DNA synthesis seems to occur after the formation of deoxynucleoside triphosphates, as opposed to the inhibition of precursor biosynthesis.

Other sesquiterpene lactones studied by us were also found to inhibit incorporation of [^{3}H]thymidine into DNA in system B. Assuming that these compounds, like parthenolide, do not affect phosphorylation of thymidine to dTTP, the observed reduction of thymidine incorporation could be considered as a measure of inhibition of DNA synthesis. It should be noted that the most potent inhibitory properties in this assay were shown by the lactones which were reported to exhibit antitumor activity *in vivo*, namely, elephantopin (1), vernolepin (1), eupatoriopicrin (22), and alatolide (22). Moreover, the ability to inhibit DNA synthesis seems to be correlated with the cytotoxicity of sesquiterpene lactones against HeLa cells. The correlation found was statistically significant, although not strong. However, ED_{50} values (cytotoxic activity) were determined after 72 hr of incubation of cells with inhibitors, whereas ID_{50} values (ability to inhibit DNA synthesis) were obtained after only 20 min of incubation. The difference in time of exposure of cells to inhibitor could also explain why the observed ID_{50} values were much higher than ED_{50} values. On the other hand, the extent of the effects of sesquiterpene lactones may depend on cell density, since the amounts of particular lactones inhibiting DNA biosynthesis by 50% are of the same order of magnitude as those inhibiting cell growth by 50%, when both values are expressed as moles per cell rather than as molar concentrations.

Why epitulipinolide (Compound VI) showed little ability to inhibit DNA biosynthesis, as compared with its high cytotoxic activity, remains unknown but could be due to slow penetration into cells and/or to slow activation of this lactone.

As hitherto discussed, the ability to inhibit DNA synthesis appeared to be a common feature among cytotoxic and antitumor sesquiterpene lactones that seems to play an important role in the biological activity of these compounds. Hence, in contrast to the currently accepted viewpoint (see Introduction), our results suggest DNA as a target molecule for sesquiterepene lactones and are consistent with the hypothesis that alkylation of DNA represents a molecular basis of cytotoxic action of these compounds. Other possibilities cannot be excluded as yet, although they seem less likely.

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Send reprint requests to: Dr. Jerzy Konopa, Department of Pharmaceutical Technology and Biochemistry, Technical University of Gdansk, 80–952 Gdansk, Poland.