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Antitumor Agents XXIX: Effects of Eupahyssopin on Nucleic Acid, Protein, Anaerobic, and Aerobic Metabolism of Ehrlich Ascites Tumor Cells

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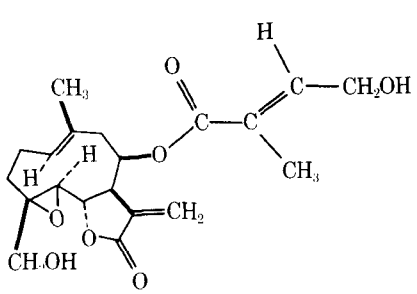
Abstract □ Evidence is presented that the antitumor agent eupahyssopin, a germacranolide, inhibits deoxyribonucleic acid, ribonucleic acid, protein, and cholesterol synthesis of Ehrlich ascites tumor cells in CF_1 male mice. Eupahyssopin appears to bind to nucleotide bases of deoxyribonucleic acid. The drug also inhibits deoxyribonucleic acid and messenger ribonucleic acid polymerase activities and, marginally, thymidylate synthetase activity. Template activity, as regulated by phosphorylation of histones and nonhistones, and cyclic 3′,5′-adenosine monophosphate levels also were affected by drug therapy. Lysosomal enzymes, *e.g.*, deoxyribonuclease and ribonuclease, and cathepsin activities were inhibited by eupahyssopin administration. A number of sulfhydryl-bearing enzymes of the glycolytic and Krebs cycles were inhibited by drug treatment, as were enzymes required for glycogen synthesis and breakdown and for phosphatase hydrolysis. Eupahyssopin also inhibited both basal respiration and coupled oxidative phosphorylation processes of the tumor cell. It was proposed that eupahyssopin, which contains the O=CC=CH_2 as an α-methylene-γ-lactone moiety within its allylic ester side chain, has the ability to undergo a rapid Michael-type addition with sulfhydryl groups of these enzymes and thus to inhibit their activities in a manner analogous to that described for other sesquiterpene antitumor agents containing the O=CC=CH_2 moiety.

Keyphrases □ Eupahyssopin—effects on nucleic acid, protein, anaerobic, and aerobic metabolism of Ehrlich ascites tumor cells □ Metabolism—nucleic acid, protein, anaerobic, and aerobic, in Ehrlich ascites tumor cells, effects of eupahyssopin □ Enzyme activity—Ehrlich ascites tumor cells, effects of eupahyssopin □ Antineoplastic agents, potential—eupahyssopin, effects on nucleic acid, protein, anaerobic, and aerobic metabolism of Ehrlich ascites tumor cells

A new germacranolide, eupahyssopin (I), was isolated from the chloroform extract of the whole plant *Eupatorium hyssopifolium* (1). Its stereochemistry and physical characteristics previously were reported (2). As an antitumor agent, eupahyssopin is active in the Walker 256 carcinosarcoma Sprague–Dawley rat survival system at 2.5 mg/kg/day (T/C = 330), in the P-388 lymphocytic leukemia screen in DBA/2 mice at 25 mg/kg/day (T/C = 132), and in the Lewis lung screen in $C_{57}B1_6$ mice at 25 mg/kg/day (T/C = 147) and caused 93% inhibition of Ehrlich ascites cell growth in CF_1 mice at 25 mg/kg/day (3). The effects of eupahyssopin on nucleic acid, chromatin protein, protein, anaerobic and aerobic glycolysis, and oxidative phosphorylation processes of Ehrlich ascites tumor cells are now reported.



I

EXPERIMENTAL

Male CF_1 mice, ~30 g, were implanted intraperitoneally on Day 0 with 10^6 Ehrlich ascites tumor cells. On Days 7, 8, and 9, the animals were treated with a subacute dose, 0.25 mg ip, of eupahyssopin suspended by homogenization in 0.05% polysorbate 80–water. Animals were sacrificed on the 10th day, and the ascites fluid was collected from the peritoneal cavity. *In vitro* oxidative phosphorylation studies were determined on untreated, harvested, Day 10 Ehrlich ascites cells. The number of tumor cells per milliliter and the 0.4% trypan blue uptake were determined with a hemocytometer.

Incorporation of thymidine into deoxyribonucleic acid was determined by the method of Chae *et al.* (4). One hour prior to animal sacrifice, 10 μCi of [^{14}C-methyl]-thymidine (54 mCi/mmole) was injected intraperitoneally. The deoxyribonucleic acid was isolated, and the carbon-14 content was determined in two parts of toluene, one part of octoxynol, 0.4% 2,5-diphenyloxazole, and 0.01% 1,4-bis[2-(5-oxazolyl)]benzene scintillation fluid and corrected for quenching.

The deoxyribonucleic acid concentration was determined by UV spectrophotometry at 260 nm, using calf thymus deoxyribonucleic acid as a standard. Uridine incorporation into ribonucleic acid was determined in an analogous manner, utilizing 10 μCi of 5-^{3}H-uridine (24.2 Ci/mmole). Ribonucleic acid was extracted by the method of Wilson *et al.* (5). Leucine incorporation into protein was determined by the method of Sartorelli (6), using 1 μCi of 1-^{14}C-leucine (56.9 mCi/mmole). The effects of eupahyssopin on 1-^{14}C-acetic acid (57.8 mCi/mmole) incorporation into cholesterol of Ehrlich ascites cells also were measured (7).

Nuclear deoxyribonucleic acid polymerase activity was determined on isolated nuclei (8). The incubation medium was that of Sawada *et al.* (9), except that 2-^{14}C-deoxyribothymidine triphosphate (45 mCi/mmole) was used and the insoluble nucleic acids were collected on glass fiber paper (GF/F) by vacuum suction. Deoxythymidylate monophosphate and diphosphate kinase activities were determined by the method of Maley and Ochoa (10), which is based on the disappearance of 0.1 μmole of reduced nicotinamide adenine dinucleotide at 340 nm in 20 min. Thymidylate synthetase activity was assayed by the method of Kampf *et al.* (11), utilizing a postmitochondrial (9000×*g* for 10 min) and 5 μCi of 5′-^{3}H-deoxyuridine monophosphate (11 Ci/mmole).

Phosphorylation of histones was determined by injecting intraperitoneally 10 μCi of γ-^{32}P-adenosine triphosphate (7.97 Ci/mmole) 1 hr prior to sacrifice. The nuclei were isolated (8), and the histone chromatin

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protein was extracted by the method of Raineri *et al.* (12). Nonhistone chromatin phosphorylation by a nucleus protein kinase (13) was determined on isolated nuclei (8), utilizing 2 nmoles of γ-^{32}P-adenosine triphosphate (7.97 Ci/mmole). Chromatin protein was collected on nitrocellulose membrane filters (13).

Cyclic 3′,5′-adenosine monophosphate levels were determined by the method of Gilman (14), using ^{3}H(*G*)-cyclic 3′,5′-adenosine monophosphate (39.8 Ci/mmole). Messenger, ribosomal, and hormone sensitive ribonucleic acid polymerase activities were measured by the methods of Anderson *et al.* (15) and Hall *et al.* (16) with 5,6-^{3}H-uridine 5′-triphosphate (35 Ci/mmole). Ammonium sulfate concentrations of 0.3, 0.04, and 0.0 *M* were employed to measure the respective types of ribonucleic acid polymerase activity. Insoluble ribonucleic acid was collected on glass fiber paper (GF/F) by vacuum suction.

The effect of eupahyssopin on hydrolytic enzymes also was determined. Deoxyribonuclease activity was measured at pH 5.0 by a modification of the deDuve method (16). Ribonuclease activity and acid cathepsin activity were determined at pH 5.0 by the methods of Cho-Chung and Gullino (17).

In vitro UV binding studies (18) were conducted with eupahyssopin (0.11 μg/ml) and deoxyribonucleic acid (38 μg/ml) or deoxyriboguanosine monophosphate (38 μg/ml) in 0.1 *M* phosphate buffer, pH 7.2, over a range of 200–340 nm for 24 hr.

The following glycolytic enzymatic activities were determined for both control and treated cells: hexokinase (19), phosphofructokinase (20), aldolase (21), lactic dehydrogenase (22), glucose-6-phosphatase (23), fructose-1,6-diphosphatase (24), phosphorylase *a*, (24), and glycogen synthetase (25), utilizing ^{14}C(*U*)-uridine diphosphate-D-glucose (240 mCi/mmole).

The Krebs cycle dehydrogenase activities of succinic (26) and malic (27) dehydrogenases also were determined. Inorganic phosphate was determined by the method of Chen *et al.* (28), and protein was determined by the method of Lowry *et al.* (29). *In vitro* effects of eupahyssopin (0.2 mg) on Ehrlich ascites tumor cell oxidative phosphorylation were measured in the presence and absence of adenosine diphosphate, utilizing succinate, a flavin adenine dinucleotide-linked dehydrogenase, or α-ketoglutarate, a nicotinamide adenine dinucleotide-linked dehydrogenase, as the substrate (24).

RESULTS

The normal number of Ehrlich ascites cells per milliliter on Day 10 was 223×10^{6}. Eupahyssopin treatment at the subacute dose of 0.25 mg on Days 7, 8, and 9 reduced the cell count by 61%. Drug treatment resulted in no significant difference in the percentage of nonviable cells or in the size or general morphology of the cell as observed by gross microscopy.

For the control animal, the thymidine incorporation into deoxyribonucleic acid was 241,675 dpm/mg of deoxyribonucleic acid. Drug treatment inhibited deoxyribonucleic acid synthesis 69%. Uridine incorporation into ribonucleic acid for the 10-day Ehrlich ascites cells was 39,525 dpm/mg of ribonucleic acid. Eupahyssopin treatment reduced ribonucleic acid synthesis 52%. Leucine incorporation into protein for the control was 4213 dpm/mg of protein. This amount was decreased 42% by drug treatment. Acetic acid incorporation into cholesterol for membrane synthesis for the control was 273,271 dpm/mg of protein. Drug treatment reduced cholesterol synthesis by 76%.

A more detailed examination was made of the enzymes required for the synthesis of deoxyribonucleic acid synthetase. Nuclear deoxyribonucleic acid polymerase activity alpha, beta, and gamma for the control was 7561 dpm/mg of protein. Drug treatment resulted in a 71% reduction in deoxyribonucleic acid polymerase activity. Deoxythymidylate monophosphate kinase activity for the control was 0.413 O.D. (change in absorbance) unit/mg of protein/20 min, and deoxythymidylate diphosphate kinase activity was 0.557 O.D. unit/mg of protein/20 min. Eupahyssopin treatment caused 16 and 12% reductions in deoxynucleotide kinase activities, respectively, which were not significant. Thymidylate synthetase activity for 10-day Ehrlich ascites cells was 103,328 dpm/mg of protein, which was reduced 37% by drug treatment.

In vitro binding studies with deoxyribonucleic acid or deoxyguanosine monophosphate indicated that eupahyssopin interacted with nucleotide bases. Eupahyssopin has an absorption peak at 219–222 nm. After reacting eupahyssopin with deoxyribonucleic acid or deoxyguanosine monophosphate, the absorption peak of eupahyssopin at 219–222 nm immediately disappeared; during 2–20 hr, the magnitude of the absorption of deoxyribonucleic acid at 260 nm or of deoxyguanosine monophosphate at 257 nm increased moderately. Incubation of the drug alone over 24 hr resulted in no change in absorption properties, *e.g.*, magnitude and shift, indicating that the drug was not decomposing.

Messenger (nuclei) ribonucleic acid polymerase activity for the control was 120,811 dpm/mg of protein, whereas ribosomal (nucleolus) ribonucleic acid polymerase activity was 65,460 dpm/mg of protein and hormone-sensitive ribonucleic acid polymerase activity was 46,830 dpm/mg of protein. Treatment with eupahyssopin resulted in a 55% reduction, a 4% reduction, and a 5% increase, respectively, in ribonucleic acid polymerase activities.

Phosphorus incorporation into histone protein for 10-day normal Ehrlich ascites cells was 2954 dpm/mg of chromatin protein, which was reduced 51% by eupahyssopin. Phosphorus incorporation into nonhistone chromatin protein was 9803 dpm/mg of protein. Drug administration reduced the activity of this chromatin protein kinase by 31%. The cyclic 3′,5′-adenosine monophosphate level in the control was 3.65 pmoles/10^{6} cells. Eupahyssopin treatment elevated this cyclic nucleotide level 114%.

The catabolic rate of nucleic acids and proteins also was determined to eliminate the possibility that the drug accelerated hydrolytic enzymatic activity and thus indirectly lowered the incorporation of precursors into nucleic acids and proteins. Deoxyribonuclease activity for the 10-day Ehrlich ascites cells was 35 μg of deoxyribonucleic acid hydrolyzed/mg of protein/30 min, which was inhibited 69% by eupahyssopin administration. Ribonuclease activity for the control was 24 μg of ribonucleic acid hydrolyzed/mg of protein/30 min. Drug treatment reduced this amount 61%. Ten-day tumor cell cathepsin activity was 0.90 mg of protein hydrolyzed/mg of protein/30 min. Eupahyssopin treatment reduced cathepsin activity 83%.

Hexokinase activity for 10-day Ehrlich ascites tumor cells was calculated as a change in absorbance equal to 0.260 O.D. unit/mg of protein/min, based on 500 μmoles of nicotinamide adenine dinucleotide phosphate, which was suppressed 59% by eupahyssopin treatment. Phosphofructokinase activity for the control was 0.176 O.D. unit/mg of protein/min based on 1.125 μmole of reduced nicotinamide adenine dinucleotide; administration of eupahyssopin reduced phosphofructokinase activity 10%. Aldolase activity for the control was 302 mU/mg of protein, which was suppressed 42% by eupahyssopin. Lactic dehydrogenase activity for Ehrlich ascites tumor cells was 0.623 O.D. unit/mg of protein/min based on 0.12 μmole of reduced nicotinamide adenine dinucleotide. Eupahyssopin reduced the activity of this dehydrogenase 50%.

Glucose-6-phosphatase activity for the control was 11.24 μg of phosphate released/mg of protein/15 min. Administration of eupahyssopin reduced glucose-6-phosphatase activity 85%. Fructose-1,6-diphosphatase activity was 29.82 μg of phosphate released/mg of protein/15 min. Drug therapy resulted in 54% reduction. Phosphorylase *a* activity for the control was 5.72 μg of phosphate taken up from the medium/mg of protein/hr; drug treatment reduced phosphorylase *a* activity 58%. Glycogen synthetase activity for the control was 1540 dpm/mg of protein; eupahyssopin reduced glycogen synthesis 57%.

Succinic dehydrogenase activity for the control was 0.069 O.D. unit/mg of protein/min based on 15 μmoles of potassium ferricyanide, which eupahyssopin administration reduced 41%. Malic dehydrogenase activity for 10-day Ehrlich ascites cells was 0.80 O.D. unit/mg of protein/min based on 15 μmoles of reduced nicotinamide adenine dinucleotide. Malic dehydrogenase was suppressed 55% by eupahyssopin treatment.

The basal respiration (state 4) of 10-day Ehrlich ascites tumor cells with sodium succinate as the substrate was 5.273 μl of oxygen/mg of protein/hr; in the presence of adenosine diphosphate (state 3), stimulated respiration or coupled oxidative phosphorylation was 8.752 μl of oxygen/mg of protein/hr. *In vitro* eupahyssopin suppressed state 4 respiration 18% and state 3 respiration 13%. Use of sodium α-ketoglutarate as the substrate resulted in a state 4 respiration of 3.569 μl of oxygen/mg of protein/hr and in a state 3 respiration of 5.156 μl of oxygen/mg of protein/hr. Eupahyssopin inhibited state 4 respiration 41% and state 3 respiration 57% (Tables I and II). The probable significance level (p) was determined by the Student t test (30).

DISCUSSION

Eupahyssopin administration significantly inhibited the synthesis of deoxyribonucleic acids, ribonucleic acids, proteins, and cholesterol in 10-day Ehrlich ascites tumor cells. The synthesis of all of these macromolecules is required for rapidly proliferating cells. One explanation for the reduction of radiolabeled precursor into these macromolecules is that drug treatment accelerated catabolism of the macromolecule. Administration of eupahyssopin did not accelerate the hydrolytic activity of enzymes responsible for catabolism of these macromolecules; rather, the

Table I—*In Vivo* Effects of Eupahyssopin on Nucleic Acid and Protein Metabolism of Ehrlich Ascites Tumor Cells (n = 6)

Parameter	Control, 0.05% Polysorbate 80, $\bar{x} \pm SD$	Percent Control, Treated on Days 7, 8, and 9 with 0.25 mg of Eupahyssopin, $\bar{x} \pm SD$
Thymidine incorporation into deoxyribonucleic acid	100 ± 21	31 ± 13[a]
Uridine incorporation into ribonucleic acid	100 ± 18	48 ± 15[a]
Leucine incorporation into protein	100 ± 13	58 ± 11[a]
Acetic acid incorporation into cholesterol	100 ± 23	24 ± 8[a]
Deoxyribonuclease activity	100 ± 19	31 ± 5[a]
Ribonuclease activity	100 ± 22	39 ± 20[a]
Cathepsin activity	100 ± 19	17 ± 18[a]
Deoxyribonucleic acid polymerase activity	100 ± 5	29 ± 5[a]
Thymidylate monophosphate kinase activity	100 ± 15	84 ± 9
Thymidylate diphosphate kinase activity	100 ± 8	88 ± 22
Thymidylate synthetase activity	100 ± 13	63 ± 7[a]
Messenger ribonucleic acid polymerase activity	100 ± 18	45 ± 16[a]
Ribosomal ribonucleic acid polymerase activity	100 ± 6	96 ± 4
Hormone sensitive ribonucleic acid polymerase activity	100 ± 11	105 ± 20
Phosphorylation of histones	100 ± 8	49 ± 3[a]
Phosphorylation of nonhistones	100 ± 3	69 ± 5[a]
Cyclic 3′,5′-adenosine monophosphate levels	100 ± 5	214 ± 3[a]
Number of tumor cells per milliliter of ascites fluid	100 ± 19	39 ± 10[a]

[a] p = 0.001.

drug inhibited significantly deoxyribonuclease, ribonuclease, and cathepsin activities.

The major site of eupahyssopin inhibition in deoxyribonucleic acid synthesis was the deoxyribonucleic acid polymerase nuclei enzymes, whereas the thymidylate synthetase enzyme appeared to be reduced marginally. The deoxyribonucleotide kinase enzymes were essentially unaffected by drug treatment. Previously (18), it was demonstrated that compounds containing the O=CC=CH_2 system were capable of undergoing a rapid Michael-type addition with biological nucleophiles, *e.g.*, sulfhydryl groups. Both deoxyribonucleic acid polymerase alpha and gamma of the nucleus (31) and thymidylate synthetase possess an exposed sulfhydryl group which can be alkylated (32). Contained in the structure of eupahyssopin is the O=CC=CH_2 system as an α-methylene-γ-lactone and within the allylic ester side chain. The α-methylene-γ-lactone moiety undergoes a rapid-type Michael addition with L-cysteine and reduced glutathione (18). A similar phenomenon occurs with the sesquiterpene lactones, helenalin and tenulin (18, 33).

Cellular proliferation is regulated by phosphorylation of chromatin histones, *i.e.*, the F-1 fraction, by a protein kinase (34). Increased phosphorylation occurs when cyclic- 3′,5′-adenosine monophosphate levels are decreasing and deoxyribonucleic acid polymerase enzymes are increasing in a concentration that would require protein synthesis, *i.e.*, G_1 or early S phase of the cell cycle. Eupahyssopin treatment inhibited phosphorylation of histones and protein synthesis and significantly elevated cyclic-3′,5′-adenosine monophosphate levels. Increased intracellular levels of this cyclic nucleotide are associated with reversal of tumor morphology (35), reduced cellular proliferation, and enhanced cellular differentiation (36).

Nonhistone chromatin protein phosphorylation fluctuates during the cell cycle and parallels ribonucleic acid transcription and cellular differentiation (37). However, in rapidly proliferating lymphocytes, the phosphorylation of a particular fraction (mol. wt. 52,000) was inhibited when cyclic 3′,5′-adenosine monophosphate was present in a high concentration. Inhibition of the phosphorylation of this fraction correlates with the inhibition of cellular proliferation (38). Eupahyssopin treatment inhibited phosphorylation of nonhistone chromatin protein. Other sesquiterpene lactones inhibited chromatin phosphorylation and elevated cyclic 3′,5′-adenosine monophosphate concentrations in tumor cells (33).

Table II—Effects of Eupahyssopin on Anaerobic and Aerobic Metabolism of Ehrlich Ascites Cells

Parameter	Control 0.05% Polysorbate 80, $\bar{x} \pm SD$	Percent Control, Treated on Days 7, 8, and 9 with 0.25 mg of Eupahyssopin, $\bar{x} \pm SD$
In Vivo Enzymatic Assay (n = 6)		
Hexokinase activity	100 ± 8	41 ± 8[a]
Phosphofructokinase activity	100 ± 39	90 ± 15
Aldolase activity	100 ± 7	58 ± 6[a]
Lactic dehydrogenase activity	100 ± 9	50 ± 12[a]
Glucose-6-phosphatase activity	100 ± 23	15 ± 9[a]
Fructose-1,6 diphosphatase activity	100 ± 7	46 ± 4[a]
Phosphorylase a activity	100 ± 15	42 ± 22[a]
Glycogen synthetase activity	100 ± 14	43 ± 10[a]
Succinic dehydrogenase activity	100 ± 16	59 ± 6[a]
Malic dehydrogenase activity	100 ± 2	45 ± 8[a]
In Vitro Oxidative Phosphorylation (n = 8)		
Succinate		
State 3	100 ± 4	87 ± 5[b]
State 4	100 ± 3	82 ± 7[b]
α-Ketoglutarate		
State 3	100 ± 3	53 ± 8[a]
State 4	100 ± 6	59 ± 14[a]

[a] p = 0.001. [b] p = 0.005.

The UV spectral studies suggest that eupahyssopin interacted with the nucleotide bases of deoxyribonucleic acid. The fact that messenger ribonucleotide polymerase activity also was suppressed by drug treatment, indicating that the transcription processes utilizing deoxyribonucleic acid as a template were impaired, supports the idea that the drug binds to nucleotide bases, particularly since rRNA polymerase activity was not affected by drug treatment, which does not require a DNA template at this time. If the drug does bind to nucleotide bases, this binding may also explain the reduction in deoxyribonucleic acid polymerase activity, which also requires an intact DNA template.

Compounds containing the α-methylene-γ-lactone moiety inhibited enzymes of the Embden–Meyerhof and Krebs cycles (24, 38–40). Eupahyssopin inhibited hexokinase, aldolase, lactic dehydrogenase, and glycogen synthetase enzymatic activities but not phosphofructokinase. These enzymes contain sulfhydryl groups (24, 38–40) which can be alkylated by the O=CC=CH_2 moiety. The activities of the hydrolytic enzymes phosphorylase a, glucose-6-phosphatase, and fructose-1,6-diphosphatase were inhibited significantly by eupahyssopin administration. Dehydrogenases of the Krebs cycles also contain sulfhydryl groups in the enzymes and iron inorganic sulfides flavin proteins as integral components of the dehydrogenase enzymes (41). These groups can be subject to alkylation. Both succinic and malic dehydrogenase activities were reduced by drug treatment.

A number of antitumor agents inhibit respiration and oxidative phosphorylation (42). A number of inhibitors of protein synthesis also suppress respiration processes (43). Eupahyssopin inhibited both basal respiration and coupled oxidative phosphorylation or mitochondrial adenosine triphosphate synthesis. This action was more obvious using α-ketoglutarate, a nicotinamide adenine dinucleotide-linked dehydrogenase that contains an iron–sulfur center (44), than with succinate, which is a flavin adenine dinucleotide-linked enzyme.

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Antitumor Agents XXX: Evaluation of α-Methylene-γ-lactone-Containing Agents for Inhibition of Tumor Growth, Respiration, and Nucleic Acid Synthesis

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Abstract □ Evidence is presented that a number of sesquiterpene lactones isolated from plants and synthesized pyrimidines containing the α-methylene-γ-lactone moiety are potent inhibitors of Walker 256 carcinosarcoma and Ehrlich ascites tumor growth and marginal inhibitors of P-388 lymphocytic leukemia and Lewis lung tumor growth. *In vitro* aerobic basal respiration and oxidative phosphorylation processes of Ehrlich ascites cells were inhibited by these agents as well as deoxyribonucleic acid polymerase and thymidylate synthetase enzymatic activities. These studies indicate that the α-methylene-γ-lactone moiety, the β-unsubstituted cyclopentenone ring, and the α-epoxycyclopentanone system are the essential moieties for inhibition of these biochemical parameters.

Keyphrases □ α-Methylene-γ-lactone-containing agents—evaluated for effect on tumor growth, respiration, and nucleic acid synthesis □ Antineoplastic activity—various α-methylene-γ-lactone-containing agents evaluated □ Enzyme activity, tumor—effect of various α-methylene-γ-lactone-containing agents evaluated □ Nucleic acid synthesis, tumor—effect of various α-methylene-γ-lactone-containing agents evaluated □ Structure–activity relationships—various α-methylene-γ-lactone-containing agents evaluated for effect on tumor growth, respiration, and nucleic acid synthesis

Recently (1–13), some sesquiterpene lactones and related analogs were isolated or synthesized. A few of these compounds were shown to be potent antitumor agents of Walker 256 ascites carcinosarcoma (Sprague–Dawley rats at 2.5 mg/kg/day) and Ehrlich ascites tumor (CF_1 male mice at 33.3 mg/kg/day) and marginally active against P-388 lymphocytic leukemia (DBA/2 mice at 25 mg/kg/day) (5, 6). Helenalin, tenulin, and eupahyssopin were

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