sulfate. Solvent removal left an oily residue, which crystallized upon trituration with petroleum ether. Recrystallization of the solid from benzene–petroleum ether yielded 7.6 g (41% yield) of VIII*a*, mp 122–124°.

5 - Diethylaminomethyl - α,α - diphenylfurfuryl Alcohol (VIII *b*)—Methyl 5-diethylaminomethyl-2-furoate (15.8 g, 75 mmoles) was reacted with phenylmagnesium bromide (300 mmoles) as in the preparation of VIII*a*. Compound VIII*b*, 10.0 g (40% yield), was obtained, mp 110–111.5° [lit. (9) mp 112–113°].

5-Dialkylaminomethyl-α,α-diphenylfurfuryl Alcohol Alkiodides (IV*a*–IV*d*)—Two grams of the tertiary amine (VIII*a* or VIII*b*) was dissolved in a minimum quantity of benzene. Methyl iodide or ethyl iodide (2 ml) was added, and the mixture was allowed to stand overnight. The product was removed by filtration and recrystallized from isopropanol. The physical properties of the compounds are given in Table I; NMR (dimethyl sulfoxide-d_6): 7.32–7.34 (s, 10H, phenyl), 6.83–6.88 (d, 1H, furan), 6.67–6.70 (s, 1H, OH), 6.17–6.27 (d, 1H, furan), and 4.59–4.68 (s, 2H, furfuryl methylene). In addition, the different compounds exhibited appropriate NMR peaks for the *N*-alkyl groups: 3.18–3.28 (m, NCH_2C), 2.91–3.04 (s, NCH_3), and 1.16–1.22 (t, CCH_3).

REFERENCES

(1) R. P. Stephenson, *Br. J. Pharmacol.*, **11**, 379 (1956).

(2) R. Schneider and A. R. Timms, *ibid.*, **12**, 30 (1957).

(3) J. M. van Rossum and E. J. Ariëns, *Arch. Int. Pharmacodyn. Ther.*, **110**, 349 (1957).

(4) E. J. Ariëns and A. M. Simonis, *Ann. N.Y. Acad. Sci.*, **144**, 842 (1967).

(5) E. J. Ariëns and A. M. Simonis, *J. Pharm. Pharmacol.*, **16**, 137 (1964).

(6) E. J. Ariëns, *Adv. Drug Res.*, **3**, 235 (1966).

(7) J. F. Moran and D. J. Triggle, in "Cholinergic Ligand Interactions," Academic, New York, N.Y., 1971, pp. 119–136.

(8) E. J. Ariëns and A. M. Simonis, *Arch. Int. Pharmacodyn. Ther.*, **127**, 479 (1960).

(9) C. L. Zirkle, U.S. pat. 3,097,206 (1963); through *Chem. Abstr.*, **59**, 13,950 (1963).

(10) R. F. Holdren and R. M. Hixon, *J. Am. Chem. Soc.*, **68**, 1198 (1946).

(11) J. F. Stubbins, P. M. Hudgins, J. Andrako, and A. J. Beebe, *J. Pharm. Sci.*, **57**, 534 (1968).

(12) O. Arunlakshana and H. O. Schild, *Br. J. Pharmacol.*, **14**, 48 (1959).

(13) P. M. Hudgins and J. F. Stubbins, *J. Pharmacol. Exp. Ther.*, **166**, 237 (1969).

(14) J. F. Stubbins, P. M. Hudgins, D. C. Murphy, and T. L. Dickerson, *J. Pharm. Sci.*, **61**, 470 (1972).

(15) British pat. 705,950 (1954); through *Chem. Abstr.*, **49**, 2233 (1955).

ACKNOWLEDGMENTS

Supported in part by National Institutes of Health Grant NS-07273.

The authors thank Ms. T. L. Dickerson for technical assistance.

Mode of Action of Sesquiterpene Lactones as Anti-Inflammatory Agents

I. H. HALL *x, C. O. STARNES, Jr. *, K. H. LEE *, and T. G. WADDELL ‡

Received July 29, 1979, from the **Division of Medicinal Chemistry, School of Pharmacy, University of North Carolina, Chapel Hill, NC 27514,* and the ‡*Department of Chemistry, University of Tennessee at Chattanooga, Chattanooga, TN 37401.* Accepted for publication December 7, 1979.

Abstract □ Sesquiterpene lactones containing an α-methylene-γ-lactone moiety were shown to be potent inhibitors of carrageenan-induced edema and chronic adjuvant-induced arthritis in rodents at 2.5 mg/kg/day. The mode of action of sesquiterpene lactones as anti-inflammatory agents appeared to be at multiple sites; for example, at 5×10^{-4} *M*, the sesquiterpene lactones effectively uncoupled the oxidative phosphorylation of human polymorphonuclear neutrophils and elevated the cyclic adenosine monophosphate levels of rat neutrophils and rat and mouse liver cells. Free and total lysosomal enzymatic activity was inhibited by these agents at 5×10^{-4} *M* in both rat and mouse liver and rat and human neutrophils. Furthermore, the structure–activity relationships for the stabilization of lysosomal membrane for rat liver cathepsin activity followed the same structural requirement necessary for anti-inflammatory activity; *i.e.*, the α-methylene-γ-lactone moiety contributed the most activity, whereas the β-unsubstituted cyclopentenone and α-epoxycyclopentanone contributed only minor activity. Human polymorphonuclear neutrophil chemotaxis was inhibited at low concentrations (*i.e.*, 5×10^{-5} and 5×10^{-6} *M*), whereas prostaglandin synthetase activity was inhibited at a higher concentration (*i.e.*, 10^{-3} *M*) by the sesquiterpene lactones.

Keyphrases □ Anti-inflammatory agents—sesquiterpene lactones, mode of action determined *in vitro* and *in vivo* □ Sesquiterpene lactones—anti-inflammatory agents, antiarthritic agents, mode of action determined in rat and human neutrophils and rat and mouse liver cells □ Oxidative phosphorylation—uncoupling effect of sesquiterpene lactones, mode of action determined in inflammation process □ Lysosomal membrane—effect of sesquiterpene lactones on membrane stability, hydrolytic enzyme release, mode of action in inflammation process □ Cyclic adenosine monophosphate—effect of sesquiterpene lactones on *in vitro* levels, role in inflammation process □ Structure–activity relationships—effect of sesquiterpene lactones on inflammation process, several modes of action determined *in vitro* and *in vivo*

The anti-inflammatory activity of sesquiterpene lactones in rodents was reported previously (1). An α-methylene-γ-lactone moiety within the structure of the pseudoguaianolide and germacranolide derivatives was required for activity against edema-induced carrageenan inflammation. Saturation of the 11,13-double bond of the methylene group of the lactone resulted in a loss of activity (1).

In the chronic adjuvant-induced arthritic screen, compounds containing the α-methylene-γ-lactone, the β-unsubstituted cyclopentenone, and α-epoxycyclopentanone afforded significant inhibitory activity at 2.5 mg/kg/day (1). In addition, these derivatives also suppressed the writhing reflex, induced pleurisy, delayed hypersensitivity, and passive cutaneous anaphylaxis and were mild immunostimulants of immunoglobulins (1). The effects of the

0022-3549/80/0500-0537$01.00/0

Table I—Anti-Inflammatory Activity of Sesquiterpene Lactones and Germacranolides in Sprague–Dawley Rats

Compound	Percent of Control: Anti-Inflammatory Screen[a], 2.5 mg/kg twice	Percent of Control: Antiarthritic Screen[b], 2.5 mg/kg/day
I Helenalin	25 ± 15[c]	23 ± 4[c]
II Tenulin	84 ± 18	47 ± 8[c]
III Isotenulin	84 ± 17	47 ± 10[c]
IV Aromaticin	65 ± 6[c]	—
V 2,3-Dihydrohelenalin	53 ± 19[c]	—
VI 2,3-Dihydrotenulin	93 ± 8	92 ± 5
VII 2,3-Dihydroisotenulin	92 ± 15	—
VIII 2,3-Epoxyhelenalin	54 ± 3[c]	54 ± 6[c]
IX 2,3-Epoxytenulin	57 ± 19[c]	33 ± 5[c]
X 2,3-Epoxyisotenulin	69 ± 9[c]	37 ± 3[c]
XI 2,3,11,13-Tetrahydrohelenalin	96 ± 15	—
XII α-Methylene-γ-lactone	64 ± 13[c]	17 ± 3[c]
XIII 2-Cyclopentenone	77 ± 13[d]	—
XIV Eupaformosanin	69 ± 8[c]	—
XV Eupahyssopin	43 ± 6[c]	34 ± 5[c]
XVI Molephantinin	68 ± 17[d]	—
XVII Molephantin	67 ± 8[c]	40 ± 6[c]
XVIII Deoxyelephantopin	49 ± 11[c]	31 ± 4[c]
XIX Phantomolin	54 ± 19[c]	—
XX Eupatolide	70 ± 12[d]	70 ± 7[c]
XXI Indomethacin (10 mg/kg)	27 ± 6[c]	55 ± 5[c]
XXII 0.05% Polysorbate 80	100 ± 12 (0.655 g)	100 ± 9 (0.830 g)

[a] Expressed as mean ± *SD*, $n = 8$. [b] Expressed as mean ± *SD*, $n = 6$. [c] $p \leq 0.001$. [d] $p \leq 0.010$.

sesquiterpene lactones on metabolic events associated with the inflammation process are reported here.

EXPERIMENTAL

Test Compounds—Twenty sesquiterpene lactones and related compounds were chosen. Some of the compounds were natural products isolated from plant species by literature techniques (2): helenalin (I) from *Balduina angustifolia* (3); tenulin (II) (4) and aromaticin (IV) (4, 5) from *Helenium amarum;* eupaformosanin (XIV) from *Eupatorium formosanum* (6); eupahyssopin (XV) from *E. hyssopifolium* (7); molephantinin (XVI) (8), molephantin (XVII) (9), and phantomolin (XIX) (10) from *Elephantopus mollis;* deoxyelephantopin (XVIII) from *El. carolinianus* (11); and eupatolide (XX) from *E. formosanum* (12).

2,3-Dihydrohelenalin (V), 2,3-epoxyhelenalin (VIII), and 2,3,11,13-tetrahydrohelenalin (XI) were modified chemically from helenalin (13). 2,3-Dihydrotenulin (VI), 2,3-epoxytenulin (IX), isotenulin (III), 2,3-dihydroisotenulin (VII), and 2,3-epoxyisotenulin (X) were prepared by a literature method (14), as was α-methylene-γ-lactone (XII) (15). The 2-cyclopentenone[1] and indomethacin[2] were purchased commercially.

Anti-Inflammatory Screen—Sprague–Dawley male rats (~160 g) were administered test compounds at 2.5 mg/kg ip in 0.05% polysorbate 80–water 3 hr and 30 min prior to injection of 0.2 ml of 1% carrageenan in 0.9% saline into the plantar surface of the right hindfoot. Isotonic saline was injected into the left hindfoot, which served as a baseline. After 3 hr, both feet were excised at the tibiotarsal (ankle) joint according to a modified method (16, 17), resulting in an average net increase of 0.655 g for the control animals.

Antiarthritic Screen—Male Sprague–Dawley rats (~160 g) were injected at the base of the tail with 0.2 ml of light mineral oil containing 1 mg of dried *Mycobacterium butyricum* and 0.4 mg of digitonin. The test drugs were administered on Days 3–20 at 2.5 mg/kg/day ip. Animals were sacrificed on Day 21, and the feet were excised and weighed (18). The control animals achieved an average net weight increase of 0.830 g.

Cell Preparation—Metabolic studies were conducted on CF_1 male mouse (~30 g) and Sprague–Dawley male rat (~180 g) liver homogenates (10%) in 0.25 *M* sucrose and 0.001 *M* ethylenediaminetetraacetic acid at pH 7.2. Polymorphonuclear neutrophils were collected from the peripheral blood of healthy human males (25–28 years) and separated by Ficol–Hypaque density gradient centrifugation by the technique of English and Anderson (19). The neutrophils were aspirated from the lower density gradient level; after water lysis of the red blood cells, the neutrophils constituted 99.9% of the cells.

Polymorphonuclear neutrophils were collected from the peritoneal cavity of male Sprague–Dawley rats (~280 g) ~4 hr after injection of 35 ml of 0.5% oyster glycogen in isotonic saline. The neutrophils were centrifuged at 800×*g* for 20 min, washed, and resuspended in pH 7.4 minimum essential medium and 10% fetal calf serum (20). The neutrophil concentration was ~80% of the cell content.

Oxidative Phosphorylation Studies—Basal and adenosine diphosphate-stimulated respiration of the 10% liver homogenates of rats and mice or 10^6 human polymorphonuclear neutrophils was measured using succinate or α-ketoglutarate as the substrate (21). The reaction vessel contained sucrose (55 μmoles), potassium chloride (22 μmoles), dibasic potassium phosphate (22 μmoles), sodium succinate (90 μmoles) or α-ketoglutarate (60 μmoles), and the test compounds at 5×10^{-4} *M* in 0.05% polysorbate 80–water in a total volume of 1.8 ml.

Oxygen consumption was measured using a Clark electrode connected to an oxygraph. After the basal metabolism (state 4) level was obtained, 0.257 μmole of adenosine diphosphate was added to obtain the adenosine diphosphate-stimulated respiration rate (state 3). The respiration rate was calculated in microliters of oxygen consumed per hour per milligram of wet weight of rat or mouse liver homogenates or 10^7 human polymorphonuclear neutrophils.

In vivo oxidative phosphorylation studies were conducted on mice and rats treated with helanalin (I) at 6 mg/kg or tenulin (II), eupahyssopin (XV), and deoxyelephantopin (XVIII) at 12.5 mg/kg for 3 days. Animals were sacrificed on Day 4, and liver homogenates were prepared for the respiration studies.

Lysosomal Hydrolytic Enzymatic Activities—Free and total acid phosphatase activities were studied using 0.1 *M* β-glycerol phosphate in pH 5.0, 0.1 *M* acetate buffer on CF_1 mouse liver homogenates for 20 min or rat polymorphonuclear neutrophils for 60 min (22). The test drugs were incubated *in vitro* at 5×10^{-4} *M* in 1% carboxymethylcellulose. The total enzymatic activity was obtained by treating the liver homogenates or neutrophils with 0.02% alkylphenoxypolyethoxyethanol 100 to release the bound hydrolytic enzymes from the lysosomal membrane. The reaction was terminated with 10% trichloroacetic acid, and the mixture was centrifuged.

Inorganic phosphate was determined by the method of Chen *et al.* (23). Free, total, and percent released acid phosphatase activities were calculated after correcting for the blank values. Free and total cathepsin activities were determined in an analogous manner on mouse and rat liver homogenates and on rat polymorphonuclear neutrophils with 2% azocasein as the substrate in pH 5.0, 0.1 *M* acetate buffer (24). The supernate was assayed for acid-soluble peptide fragments at 366 nm. Free, total, and percent released cathepsin activities were calculated after correcting for the blank values.

The effects of the drugs on lysosomal membrane stabilization were studied on rat liver homogenates by incubating the test drugs (5×10^{-4} *M*) for 20 min at 37° prior to measuring the enzymatic catalytic activity (25). Free and total aryl sulfatase activities were determined on mouse liver homogenates with 0.72 μmole of *p*-nitrocatechol sulfate as the substrate in pH 5.8, 0.2 *M* acetate buffer. The reaction was conducted for 20 min at 37° and terminated with 4 *N* NaOH. The formation of 4-nitrocatechol in the supernate was measured at 510 nm spectrophotometrically (25).

Cyclic Adenosine Monophosphate Levels—Isolated rat polymorphonuclear neutrophils were incubated with the test drugs at 5×10^{-4} *M* for 1 hr at 37° in pH 7.4 minimum essential medium. The reaction was stopped with 6% trichloroacetic acid, and the cyclic adenosine monophosphate levels were determined by radioimmunoassay[3] using ^{125}I-2-*O*-succinyl cyclic adenosine monophosphate tyrosine methyl ester and cyclic adenosine monophosphate antibody (26). Results were calculated in picomoles of cyclic adenosine monophosphate per 10^7 polymorphonuclear neutrophils.

Cyclic adenosine monophosphate levels also were determined after treating the mice or rats *in vivo* with helenalin (I) at 6 mg/kg or tenulin (II), eupahyssopin (XV), and deoxyelephantopin (XVIII) at 12.5 mg/kg for 3 days. The animals were sacrificed on the 4th day, and a 10% liver homogenate was prepared for the radioimmunoassay. Results were calculated as picomoles per milligram of wet liver.

Prostaglandin Synthetase Activity—The incubation medium of Tomlinson *et al.* (27) was used to determine the [^{3}H]prostaglandin for-

[1] Aldrich.
[2] Merck Sharp & Dohme.
[3] Becton Dickinson.

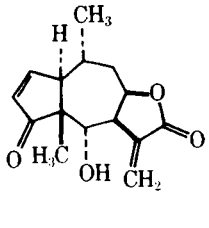
mation from [^{3}H](N)-arachidonic acid (86.2 Ci/mmole) and 10 mg of purified commercial prostaglandin synthetase from bovine seminal vesicles[4]. After 1 hr at 37°, the reaction was terminated with 1 *N* HCl, and the mixture was extracted with ether and evaporated. The residue was dissolved in ethyl acetate and spotted on silica gel TLC plates, which were eluted with chloroform–methanol–water–acetic acid (90:8:1:0.8) (28). The plates were dried and developed in iodine vapor; with the use of prostaglandin standards, the appropriate areas were scraped and counted for tritium content. Indomethacin was used as an internal standard at 10^{-4} and 10^{-6} *M*. Helenalin (I) and tenulin (II) were tested at 10^{-3} and 10^{-4} *M*.

Polymorphonuclear Neutrophil Chemotaxis—Isolated human polymorphonuclear neutrophils were utilized to measure the inhibition of chemotaxis and migration to *Escherichia coli* bacterial culture filtrate, as the chemotactant agent, by the method of Nelson *et al.* (29) on agarose tissue culture plates. The test drugs at 10^{-4}, 5×10^{-5}, and 10^{-6} *M* were incubated for 30 min at 37° with the neutrophils in pH 7.4 minimum essential medium prior to the assay. The plates were incubated in a carbon dioxide incubator at 37° for 2.5 hr and fixed in methanol overnight. After staining with Wright's stain, the migration from the wells was measured in micrometers. The migration for the control neutrophils was 991 ± 52 μm. Indomethacin was used as an internal standard at 10^{-3} *M*.

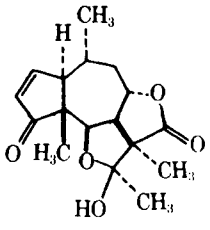
RESULTS

The sesquiterpene lactone analogs containing an α-methylene-γ-lactone moiety (I, IV, and V) possessed potent anti-inflammatory activity in rodents at a relatively low dose of 2.5 mg/kg (Table I); II, III, VI, VII, and XI were inactive. Administration of helenalin (I) resulted in the best inhibitory activity of the sesquiterpene lactones both in the carrageenan-induced screen (75%) and the chronic adjuvant-induced arthritic screen (77%). Compounds V, VIII, IX, XV, XVIII, and XIX caused 43–57% inhibition of the carrageenan-induced edema. In the chronic arthritic screen, treatment with the simple α-methylene-γ-lactone (XII) resulted in 83% inhibition. Compounds IX, X, XV, and XVIII resulted in 63–69% inhibition of induced arthritis; II, III, VIII, and indomethacin caused 45–53% inhibition of arthritis.

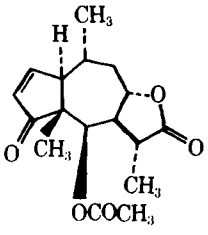
Representative compounds were selected for the biochemical studies. Helenalin (I) and tenulin (II) were selected from the pseudoguaianolides,



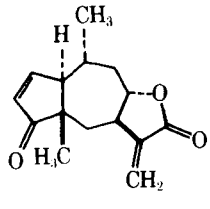
I



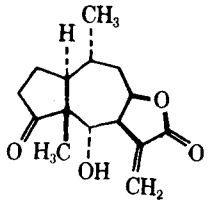
II



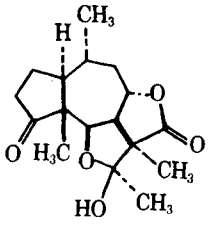
III



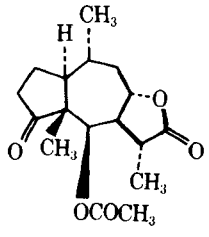
IV



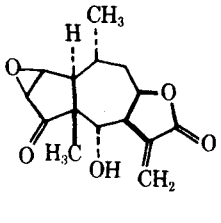
V



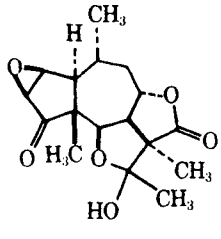
VI



VII

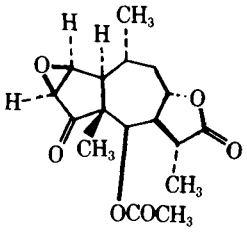


VIII

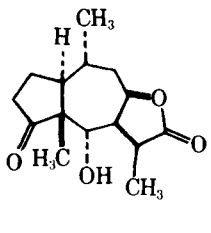


IX

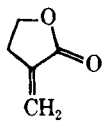
[4] Miles Research Products.



X

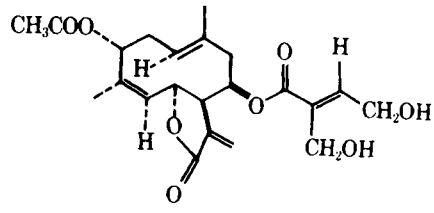


XI

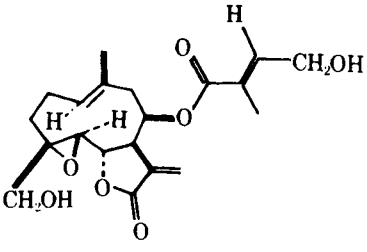


XII

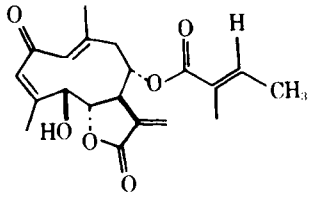
XIII



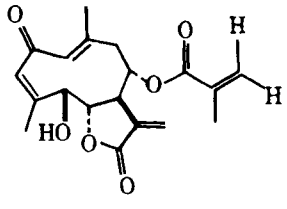
XIV



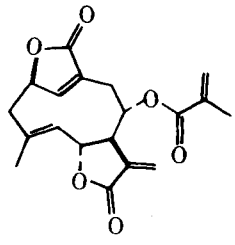
XV



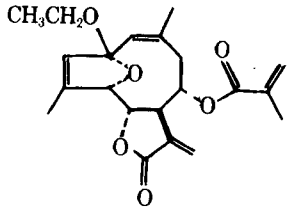
XVI



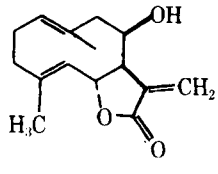
XVII



XVIII



XIX



XX

and eupahyssopin (XV) and deoxyelephantopin (XVIII) were selected from the germacranolides. The *in vitro* mouse and rat liver oxidative phosphorylation studies demonstrated that these compounds at 5×10^{-4} *M* only marginally altered the basal respiration (state 4) and the adenosine monophosphate-stimulated respiration (state 3), utilizing as the substrates either succinate, a flavin adenine dinucleotide-linked dehydrogenase, or α-ketoglutarate, a nicotinic adenine dinucleotide-linked dehydrogenase (Table II).

In vivo administration of the sesquiterpene lactones for 3 days significantly increased rat and mouse basal respiration with succinate; although not significant, the trend followed the same pattern with α-ketoglutarate. Adenosine monophosphate-stimulated respiration was reduced drastically in mouse liver with I using succinate (Table III). Human polymorphonuclear neutrophil basal respiration (Table IV) was increased significantly with I, II, XV, and XVIII at 5×10^{-4} *M* using either succinate or α-ketoglutarate as the substrate, whereas the adenosine diphosphate-stimulated respiration was reduced significantly with both succinate and α-ketoglutarate as the substrate. These studies indicate that the respiration of human neutrophils was uncoupled by these four sesquiterpene lactones.

Table II—*In Vitro* Effects of Sesquiterpene Lactones on Rodent Liver Oxidative Phosphorylation

Compound (5×10^{-4} *M*)	Percent of Control[a]			
	Succinate		α-Ketoglutarate	
	State 4	State 3	State 4	State 3
		Mouse Liver[b]		
I	94.3 ± 7.1	98.3 ± 11.7	93.2 ± 17.3	85.6 ± 9.7
II	88.3 ± 7.0	98.5 ± 11.3	95.7 ± 21.3	98.6 ± 17.4
XV	95.7 ± 6.8	97.6 ± 12.1	101.3 ± 11.7	86.7 ± 10.3
XVIII	90.1 ± 4.2	98.7 ± 9.9	99.1 ± 16.2	89.2 ± 12.1
0.05% Polysorbate 80	100.0 ± 8.8	100.0 ± 17.5	100.0 ± 6.3	
		Rat Liver[c]		
I	96.4 ± 6.2	97.2 ± 7.9	94.6 ± 12.1	88.9 ± 9.2
II	92.3 ± 7.1	96.4 ± 8.1	93.3 ± 10.8	96.7 ± 12.2
XV	101.3 ± 5.8	85.6 ± 7.6	96.5 ± 11.1	88.2 ± 10.1
XVIII	97.5 ± 3.2	88.4 ± 5.8	103.2 ± 9.1	83.6 ± 9.8
0.05% Polysorbate 80	100.0 ± 7.6	100.0 ± 7.8	100.0 ± 8.9	100.0 ± 11.4

[a] See Table III for control values. [b] Expressed as mean ± *SD*, $n = 9$. [c] Expressed as mean ± *SD*, $n = 6$.

Table III—Oxidative Phosphorylation Respiration of Rodent Liver after 3 Days of Treatment with Sesquiterpene Lactones

Compound and Dose	Percent of Control			
	Succinate		α-Ketoglutarate	
	State 4	State 3	State 4	State 3
		Mouse Liver[a]		
I, 6 mg/kg/day	158 ± 21[b]	60 ± 15[b]	108 ± 12	84 ± 23
II, 12 mg/kg/day	143 ± 19[b]	118 ± 23	113 ± 25	132 ± 15[b]
XV, 12 mg/kg/day	138 ± 17[b]	82 ± 17[c]	115 ± 19	87 ± 19
XVIII, 12 mg/kg/day	127 ± 12[c]	89 ± 11	103 ± 10	86 ± 13
0.05% Polysorbate 80	100 ± 16[d]	100 ± 12[e]	100 ± 17[f]	100 ± 6[g]
		Rat Liver[h]		
I, 6 mg/kg/day	138 ± 11[b]	84 ± 7[c]	115 ± 13	83 ± 8[c]
II, 12 mg/kg/day	125 ± 9[c]	96 ± 8	110 ± 12	95 ± 12
XV, 12 mg/kg/day	132 ± 14[c]	82 ± 5[c]	119 ± 10	86 ± 7
XVIII, 12 mg/kg/day	128 ± 13[c]	93 ± 6	99 ± 9	84 ± 10
0.05% Polysorbate 80	100 ± 12[i]	100 ± 9[j]	100 ± 13[k]	100 ± 10[l]

[a] Expressed as mean ± *SD*, $n = 8$. [b] $p \leq 0.001$. [c] $p \leq 0.010$. [d] 5.92 μl of oxygen consumed/hr/mg of wet tissue. [e] 11.31 μl of oxygen consumed/hr/mg of wet tissue. [f] 3.51 μl of oxygen consumed/hr/mg of wet tissue. [g] 5.21 μl of oxygen consumed/hr/mg of wet tissue. [h] Expressed as mean ± *SD*, $n = 6$. [i] 4.52 μl of oxygen consumed/hr/mg of wet tissue. [j] 9.13 μl of oxygen consumed/hr/mg of wet tissue. [k] 3.42 μl of oxygen consumed/hr/mg of wet tissue. [l] 6.36 μl of oxygen consumed/hr/mg of wet tissue.

Table IV—*In Vitro* Effects of Sesquiterpene Lactones on Human Polymorphonuclear Neutrophil Oxidative Phosphorylation Processes

Compound	Percent of Control[a]			
	Succinate		α-Ketoglutarate	
	State 4	State 3	State 4	State 3
I	141 ± 15[b]	59 ± 12[b]	146 ± 14[c]	67 ± 8[c]
II	166 ± 24[c]	72 ± 8[b]	141 ± 13[b]	81 ± 15
XV	149 ± 14[c]	64 ± 6[c]	149 ± 23[b]	69 ± 9[c]
XVIII	139 ± 10[b]	64 ± 12[b]	140 ± 20[d]	60 ± 11[b]
0.05% Polysorbate 80	100 ± 19[e]	100 ± 16[f]	100 ± 17[g]	100 ± 15[h]

[a] Expressed as mean ± *SD*, $n = 9$. [b] $p \leq 0.005$. [c] $p \leq 0.001$. [d] $p \leq 0.010$. [e] 3.008 ± 0.562 μl of oxygen consumed/min/10^6 cells. [f] 4.545 ± 0.722 μl of oxygen consumed/min/10^6 cells. [g] 2.632 ± 0.460 μl of oxygen consumed/min/10^6 cells. [h] 3.487 ± 0.517 μl of oxygen consumed/min/10^6 cells.

Liver hydrolytic enzyme studies *in vitro* showed that the sesquiterpene lactones were potent inhibitors of lysosomal enzyme activities (Table V). Helenalin (I) and tenulin (II) suppressed mouse liver free acid phosphatase, cathepsin, and aryl sulfatase activities at pH 5.0 and 5×10^{-4} *M*. The total enzymatic activities were suppressed by I for acid phosphatase and cathepsin, whereas the total cathepsin activity was inhibited by II. Both I and II reduced the percent of cathepsin and aryl sulfatase enzymes released into the cytoplasm.

The structure–activity study of sesquiterpene lactones *in vitro* on rat liver free cathepsin and the percent of cathepsin released demonstrated that all of the sesquiterpene lactones tested were active at 5×10^{-4} *M* (Table VI). Helenalin caused a maximum inhibition of 92%, with IV, IX, X, XII, and XIV–XIX resulting in 64–88% inhibition. The internal standard, indomethacin, resulted in 33% inhibition of free cathepsin activity at 5×10^{-4} *M*. Masking of the α-methylene-γ-lactone (compare I to II) or alteration from the *cis*- to the *trans*-fused ring (compare I to IV) also resulted in loss of activity. The 2,3-epoxy derivatives (VIII–X) improved the ability to inhibit the free cathepsin activity, with the exception of the helenalin epoxy derivative (VIII). Loss or acetylation of the 6-hydroxyl group of the cycloheptane ring along with the *trans*-fused orientation of the α-methylene-γ-lactone moiety (III and IV) resulted in an even greater loss of activity. The effects of the sesquiterpene lactones on the precent release of the cathepsin enzyme from the bound lysosomal membrane demonstrated that the sesquiterpene lactones probably stabilized the membrane and reduced the release of the enzyme.

Helenalin (I) reduced the percent of lysosomal enzyme release from 24 to 2%; the reduction for XV was to 3%, and the reduction for IX, X, XIV, and XVI–XIX was to 6–8%. Compounds that were not active in the anti-inflammatory screen had minimal effects on reducing the lysosomal enzyme release (*e.g.*, II, III, VI, VII, XI, and XIII were essentially inactive). Compounds that demonstrated potent anti-inflammatory activity also significantly reduced the lysosomal enzyme release (*e.g.*, I, IV, V, VIII–X, XII, and XIV–XIX). The lysosomal enzymatic activities of rat polymorphonuclear neutrophils also were reduced by sesquiterpene lactones at 5×10^{-4} *M* (Table VII). Helenalin (I) demonstrated 93% inhibition of free acid phosphatase activity, 94% inhibition of total acid phosphatase activity, 84% inhibition of free cathepsin activity, and 73% inhibition of total cathepsin activity. Compounds III, IV, IX, X, and XIII–XVI caused 70–87% inhibition of the free acid phosphatase activity, whereas IV, VIII–X, XII, and XIV–XVIII caused 54–88% inhibition of the total acid phosphatase activity. Free cathepsin activity was suppressed 55–67% by II, IV, V, VIII, XII, XVI, XVII, XIX, and XX. Total cathepsin activity was inhibited 51–63% by IV, VIII, XIII, and XVII.

The inhibition of lysosomal enzymatic activities of rat polymorphonuclear neutrophils correlated more with the ability to inhibit chronic arthritis than the anti-inflammatory screen. Masking of the α-methylene-γ-lactone (compare I to II), loss of the free 6-hydroxyl group, or alteration from a *cis*- to a *trans*-fusion of the α-methylene-γ-lactone moiety to the cycloheptane backbone (compare I to III and IV) again reduced the ability to inhibit the free and total lysosomal enzymatic activity.

In vivo treatment with helenalin (I) at 6 mg/kg/day for 3 days resulted in a 61 and a 51% increase in mouse liver and in rat liver cyclic nucleotide levels, respectively. Tenulin (II) at 12.5 mg/kg had little effect in mouse

Table V—*In Vitro* Effects of Sesquiterpene Lactones on CF_1 Mouse Liver Lysosomal Enzymatic Activity at pH 5.0

Compound and Dose	Percent of Control[a]								
	Acid Phosphatase			Cathepsin			Aryl Sulfatase		
	Free	Total	Percent Released	Free	Total	Percent Released	Free	Total	Percent Released
I, 5×10^{-4} *M*	77 ± 10[b]	79 ± 6[c]	97 ± 18	0[c]	6 ± 4[c]	0[c]	24 ± 11[c]	100 ± 4	24 ± 4
II, 5×10^{-4} *M*	83 ± 4[b]	91 ± 8	93 ± 11	4 ± 2[c]	19 ± 4[c]	18 ± 3[c]	22 ± 9[c]	90 ± 5	25 ± 5[c]
1% Carboxymethylcellulose	100 ± 8[d]	100 ± 5[e]	100 ± 9[f]	100 ± 4[g]	100 ± 9[h]	100 ± 6[i]	100 ± 12[j]	100 ± 6[k]	100 ± 4[l]

[a] Expressed as mean ± *SD*, $n = 6$. [b] $p \leq 0.010$. [c] $p \leq 0.001$. [d] 0.753 mg of phosphate released/hr/g of wet tissue. [e] 1.701 mg of phosphate released/hr/g of wet tissue. [f] 44% released. [g] 2.688 mg of protein released/hr/g of wet tissue. [h] 7.384 mg of protein released/hr/g of wet tissue. [i] 36% released. [j] 2.237 mg of 4-nitrocatechol released/hr/g of wet tissue. [k] 12.229 mg of 4-nitrocatechol released/hr/g of wet tissue. [l] 19% released.

Table VI—Structure–Activity Relationship of Sesquiterpene Lactones on *In Vitro* Liver Cathepsin Activity of Sprague–Dawley Rats

Compound (5×10^{-4} *M*)	Cathepsin Activity[a]		
	Free Cathepsin	Percent Control Free	Percent Released
I	0.328 ± 0.068[b]	8.1 ± 1.7[b]	1.9 ± 0.4
II	2.528 ± 0.364[b]	62.5 ± 9.0[b]	15.0 ± 2.2
III	2.030 ± 0.211[b]	50.1 ± 8.5[b]	12.1 ± 1.3
IV	1.468 ± 0.154[b]	36.3 ± 3.8[b]	8.7 ± 0.9
V	1.610 ± 0.139[b]	39.8 ± 3.4[b]	9.6 ± 0.8
VI	2.879 ± 0.146[b]	71.2 ± 3.6[b]	17.1 ± 0.9
VII	2.394 ± 0.226[b]	59.2 ± 5.5[b]	14.2 ± 1.3
VIII	1.545 ± 0.198[b]	38.2 ± 4.9[b]	9.2 ± 1.2
IX	1.019 ± 0.169[b]	25.2 ± 4.2[b]	6.0 ± 1.0
X	0.966 ± 0.134[b]	23.9 ± 3.3[b]	5.7 ± 0.8
XI	2.833 ± 0.296[b]	71.3 ±7.3[b]	17.1 ± 1.8
XII	1.467 ± 0.174[b]	36.3 ± 4.3[b]	8.7 ± 1.0
XIII	2.559 ± 0.133[b]	63.3 ± 3.3[b]	15.1 ± 0.8
XIV	1.023 ± 0.190[b]	25.3 ± 4.7[b]	6.1 ± 1.1
XV	0.465 ± 0.067[c]	11.5 ± 1.7[b]	2.8 ± 0.3
XVI	1.217 ± 0.162[b]	30.1 ± 4.0[b]	7.2 ± 0.9
XVII	1.387 ± 0.254[b]	34.3 ± 6.3[b]	8.2 ± 1.5
XVIII	0.995 ± 0.123[b]	24.6 ± 3.0[b]	5.9 ± 0.7
XIX	1.266 ± 0.171[b]	31.3 ± 4.2[b]	7.5 ± 1.0
XX	2.378 ± 0.218[b]	58.8 ± 5.4[b]	14.1 ± 1.3
1% Carboxymethylcellulose	4.0439 ± 0.207[c]	100.0 ± 8.1	24.0[d] ± 1.2
Indomethacin	2.718 ± 0.246	67.2 ± 6.1	16.1 ± 1.5

[a] Expressed as mean ± *SD*, $n = 6$. [b] $p \leq 0.001$. [c] Free cathepsin = 4.0439 mg of protein released/hr/g of wet tissue. [d] Control total cathepsin = 16.845 mg of protein released/hr/g of wet tissue; therefore, percent free control = 24.

Table VII—Structure–Activity Relationship of Sesquiterpene Lactones on *In Vitro* Cathepsin and Acid Phosphatase Activities of Rat Polymorphonuclear Neutrophils at pH 5.0

Compound (5×10^{-4} *M*)	Percent of Control[a]			
	Acid Phosphatase		Cathepsin	
	Free	Total	Free	Total
I	7 ± 1[b]	6 ± 1[b]	16 ± 2[b]	27 ± 4[b]
II	41 ± 4[b]	62 ± 6[b]	45 ± 4[b]	59 ± 3[b]
III	22 ± 2[b]	57 ± 3[b]	67 ± 5[b]	65 ± 4[b]
IV	30 ± 5[b]	33 ± 6[b]	35 ± 3[b]	44 ± 2[b]
V	54 ± 4[b]	68 ± 7[b]	42 ± 5[b]	51 ± 3[b]
VI	41 ± 3[b]	82 ± 9[c]	62 ± 4[b]	59 ± 4[b]
VII	53 ± 4[b]	100 ± 5	62 ± 4[b]	71 ± 8[b]
VIII	43 ± 7[b]	46 ± 5[b]	38 ± 3[b]	48 ± 5[b]
IX	21 ± 5[b]	38 ± 5[b]	53 ± 3[b]	69 ± 6[b]
X	16 ± 2[b]	16 ± 2[b]	62 ± 4[b]	51 ± 4[b]
XI	69 ± 6[b]	77 ± 4[b]	74 ± 7[b]	72 ± 3[b]
XII	45 ± 7[b]	44 ± 2[b]	44 ± 5[b]	58 ± 4[b]
XIII	26 ± 9[b]	90 ± 7[b]	49 ± 4[b]	37 ± 5[b]
XIV	13 ± 2[b]	12 ± 3[b]	59 ± 5[b]	61 ± 3[b]
XV	17 ± 1[b]	16 ± 2[b]	51 ± 3[b]	59 ± 2[b]
XVI	21 ± 3[b]	26 ± 2[b]	33 ± 4[b]	53 ± 5[b]
XVII	32 ± 6[b]	40 ± 5[b]	42 ± 4[b]	49 ± 5[b]
XVIII	36 ± 5[b]	39 ± 3[b]	69 ± 6[b]	78 ± 6[b]
XIX	41 ± 3[b]	72 ± 3[b]	47 ± 2[b]	60 ± 4[b]
XX	47 ± 5[b]	75 ± 4[b]	42 ± 4[b]	50 ± 3[b]
1% Carboxymethylcellulose	100 ± 3[d]	100 ± 5[e]	100 ± 3[f]	100 ± 4[g]
Indomethacin	86 ± 8	100 ± 4	100 ± 1	100 ± 3

[a] Expressed as mean ± *SD*, $n = 6$. [b] $p \leq 0.001$. [c] $p \leq 0.010$. [d] 0.272 μg of phosphate released/hr/10^7 cells. [e] 0.347 μg of phosphate released/hr/10^7 cells. [f] 0.147 mg of protein released/hr/10^7 cells. [g] 0.183 mg of protein released/hr/10^7 cells.

or rat liver. Eupahyssopin (XV) and deoxyelephantopin (XVIII) followed essentially the same pattern as I but with a slightly lower magnitude (Table VIII). The cyclic adenosine monophosphate levels of rat polymorphonuclear neutrophils were elevated 158% by I (Table IX). Compounds II, IV, V, VIII, IX, and XIII elevated the cyclic nucleotide level significantly. The isotenulin series (III, VII, and X) and the germacranolides (XIV–XX) in general lowered the cyclic nucleotide levels in neutrophils.

Sesquiterpene lactones containing an α-methylene-γ-lactone or an α-epoxycyclopentanone ring successfully inhibited polymorphonuclear neutrophil chemotaxic migration (Table X). Compounds I, IV, VIII–X, XV, XVIII, and XX caused 100% inhibition or paralysis of neutrophil migration at 5×10^{-5} *M*. At a lower concentration—*viz.*, 10^{-6} *M*, I, X, XVIII, and, surprisingly, XX caused significant inhibition.

In vitro prostaglandin synthetase activity (Table XI) was suppressed 29% by I and 8% by II at 10^{-3} *M*. These two sesquiterpene lactones had no inhibitory effects at $^{-4}$ *M*. Indomethacin caused 38 and 24% inhibition at 10^{-4} and 10^{-6} *M*, respectively.

DISCUSSION

The pseudoguaianolide (I, IV, and V) and germacranolide (XIV–XX) sesquiterpene lactones at 2.5 mg/kg significantly suppressed induced inflammation in rats. In the carrageenan-induced edema screen, the α-methylene-γ-lactone moiety plays a major role in affording anti-inflammatory activity, demonstrated by these sesquiterpene lactones. In addition, the α-epoxycyclopentanone system contributed to the anti-inflammatory activity (VIII–X) of these compounds against the induced edema. In the chronic adjuvant-induced arthritic screen, not only did the α-methylene-γ-lactone moiety and the α-epoxycyclopentanone system contribute significantly to the antiarthritic activity, but the β-unsubstituted cyclopentenone ring (II and III) also instilled antiarthritic activity for the sesquiterpene lactones.

Sesquiterpene lactones affected a number of biochemical parameters that influence the inflammation process. The synthesis of the chemical mediator, prostaglandin, was suppressed in the presence of sesquiterpene lactones at 10^{-3} *M* compared to indomethacin at 10^{-4} *M* in this isolated enzyme system. Dithiothreitol and mercaptoethanol, thiol-blocking reagents, are known to inhibit prostaglandin synthesis from arachidonic acid (30). Sesquiterpene lactones have been shown to inhibit sulfhydryl-bearing enzymes, supposedly through a Michael addition of the cyclopentenone ring and the α-methylene-γ-lactone moiety (4). It was noted that saturation of the 2,3-double bond of the cyclopentenone and the 11,13-double bond of the α-methylene-γ-lactone of helenalin resulted in a loss of anti-inflammatory activity (1).

Whereas it was difficult to demonstrate the effects of sesquiterpene lactones on mouse and rat liver oxidative phosphorylation processes, these agents definitely uncoupled the *in vitro* oxidative phosphorylation respiration of polymorphonuclear neutrophils of humans. The concentration (5×10^{-4} *M*) of sesquiterpene lactones necessary to cause uncoupling compared favorably with standard anti-inflammatory agents, *e.g.*, salicylates at 10^{-3} *M* (31) and phenylbutazone at 10^{-4} *M* (32). Suppression of oxidative phosphorylation processes of neutrophils may be associated with the lack of an efficient energy-generating system necessary for leukocyte chemotaxis, microtubular contraction, and migration to the inflammation site. A relatively low concentration of sesquiterpene lactones, *i.e.*, 5×10^{-5} and 10^{-6} *M*, was required to inhibit neutrophil chemotaxis and migration to the chemoattractant *E. coli* bacterial culture filtrate. Bryant *et al.* (33) demonstrated that sodium arsenite, which uncoupled oxidative phosphorylation at 2.5×10^{-4} *M*, also inhibited neutrophil migration. Likewise, the uncouplers iodoacetamide and dinitrophenol blocked chemotaxis partially at 10^{-4} *M* (34).

Standard anti-inflammatory drugs have been shown to inhibit liver

Table VIII—Cyclic Adenosine Monophosphate Levels [a] of Rodent Liver after 3 Days of Treatment with Sesquiterpene Lactones

Compound and Dose	Mouse, pmoles/mg of wet tissue	Percent Control	Rat, pmoles/mg of wet tissue	Percent Control
I, 6 mg/kg/day	1.94 ± 0.34[b]	161 ± 28	1.12 ± 0.21[b]	151 ± 28
II, 12.5 mg/kg/day	1.35 ± 0.35	112 ± 26	0.87 ± 0.16	118 ± 22
XV, 12.5 mg/kg/day	1.66 ± 0.28[b]	137 ± 23	1.06 ± 0.19[b]	143 ± 26
XVIII, 12.5 mg/kg/day	1.52 ± 0.24[c]	126 ± 20	0.93 ± 0.14[c]	127 ± 19
0.05% Polysorbate 80	1.21 ± 0.18	100 ± 15	0.74 ± 0.12	100 ± 16

[a] Expressed as mean ± *SD*, $n = 6$. [b] $p \leq 0.01$. [c] $p \leq 0.03$.

Table IX—Cyclic Adenosine Monophosphate Levels [a] of Rat Polymorphonuclear Neutrophils 1 hr after Incubation with Sesquiterpene Lactones

Compound (5×10^{-4} *M*)	Picomoles/10^7 cells	Percent Control
I	44.0 ± 0.5	258 ± 2.8
II	24.2 ± 0.3	142 ± 1.7
III	0.5 ± 0.1	3 ± 0.5
IV	33.0 ± 0.5	194 ± 2.8
V	34.0 ± 0.3	200 ± 1.7
VI	19.2 ± 0.4	113 ± 2.2
VII	16.0 ± 0.2	94 ± 1.1
VIII	34.0 ± 0.3	200 ± 1.7
IX	29.0 ± 0.2	171 ± 1.1
X	7.8 ± 0.1	46 ± 0.5
XI	6.5 ± 0.2	38 ± 1.1
XII	19.2 ± 0.2	113 ± 1.1
XIII	29.5 ± 0.2	174 ± 1.1
XIV	12.5 ± 0.2	74 ± 1.1
XV	9.9 ± 0.1	58 ± 0.5
XVI	15.6 ± 0.2	87 ± 1.1
XVII	18.9 ± 0.2	111 ± 1.1
XVIII	10.0 ± 0.3	57 ± 1.7
XIX	0.8 ± 0.2	5 ± 1.1
XX	11 5 ± 0.3	68 ± 1.7
0.05% Polysorbate 80	17.6 ± 0.2	100 ± 1.1
Indomethacin	17.7 ± 0.3	100 ± 1.7

[a] Expressed as mean ± *SD*, $n = 4$.

Table X—*In Vitro* Effects of Sesquiterpene Lactones in Human Polymorphonuclear Neutrophil Chemotaxic Migration

Compound	Percent Inhibition[a] 10^{-6} *M*	5×10^{-5} *M*	10^{-4} *M*
I	54 ± 4	100 ± 3	100 ± 5
II	1 ± 1	39 ± 2	100 ± 4
III	—	55 ± 3	—
IV	0	100 ± 6	—
V	—	20 ± 2	—
VI	—	0	—
VII	—	0	—
VIII	0	100 ± 5	—
IX	7 ± 2	100 ± 3	—
X	39 ± 4	100 ± 5	—
XI	—	18 ± 1	—
XII	—	4 ± 1	—
XIII	—	8 ± 3	—
XIV	—	0	—
XV	—	100 ± 4	—
XVIII	46 ± 2	100 ± 6	—
XX	58 ± 3	100 ± 4	—
0.05% Polysorbate 80	0	0	0
Indomethacin[b]	—	—	—

[a] Expressed as mean ± *SD*, $n = 4$. [b] Inhibition of indomethacin at 10^{-3} *M* is 31%.

Table XI—Effects of Helenalin and Tenulin on Prostaglandin Synthetase Activity [a]

Compound and Dose	dpm[b] per Milligram of Enzyme per Hour	Percent Control
0.05% Polysorbate 80	65,640[c] ± 3938	100 ± 6
Indomethacin (10^{-4} *M*)	40,670 ± 5694[d]	62 ± 9
I, 10^{-3} *M*	46,604 ± 2064[d]	71 ± 3
II, 10^{-3} *M*	60,389 ± 5908	92 ± 8

[a] $n = 6$. [b] Disintegrations per minute. [c] 75% conversion from [^{3}H](N)-arachidonic acid. [d] $p = 0.001$.

lysosomal aryl sulfatase enzymatic activity and lysosomal rupture; *e.g.*, at 10^{-3} *M*, indomethacin, acetyl salicylate, and phenylbutazone caused 18, 13, and 42% inhibition, respectively (25). The sesquiterpene lactones, helenalin and tenulin, at 5×10^{-4} *M* suppressed liver lysosomal aryl sulfatase enzymatic activity and rupture by 75%. Higher magnitudes of inhibition were observed with helenalin and tenulin on mouse liver cathepsin activity and rupture of lysosomes. Structure–activity studies on rat liver free cathepsin activity correlated positively with the anti-inflammatory activity of the sesquiterpene lactones. Furthermore, cathepsin D enzymatic activity has been implicated in the inflammation process as causing the release of vasoamine peptides and chemotactic factors, which are chemical mediators that can attract phagocytic cells to the site of inflammation (35). Rupture of the lysosomal membrane to release hydrolytic enzymes, *i.e.*, cathepsin, appeared to be retarded drastically by the sesquiterpene lactones. Again, there was a strong positive correlation between lysosomal membrane stabilization and the anti-inflammatory activity of the sesquiterpene lactones.

A similar pattern was observed with the incubation of sesquiterpene lactones with polymorphonuclear neutrophils. Free and total acid phosphatase and cathepsin activities were inhibited significantly following generally the same structural requirements for inhibition. The neutrophils (leukocytes) supposedly contain high concentrations of hydrolytic enzymes that are released at the sites of inflammation, causing the spread of infection and digestion of tissue cellular components. Inhibition of lysosomal release and catalytic activity will reduce the spread of inflammation and tissue damage. Both acid phosphatase (36) and crude cathepsin (37) enzymatic activities can be blocked by thiol-blocking reagents.

Elevated levels of cyclic adenosine monophosphate have been correlated with the stabilization of lysosomal membranes; *e.g.*, 10^{-6} *M* cyclic adenosine monophosphate inhibited the release of β-glucuronidase from human neutrophils (38). High cyclic adenosine monophosphate levels inhibited rabbit (39) and human (40) polymorphonuclear neutrophil chemotaxis and migration. In addition, elevated levels of cyclic adenosine monophosphate or its derivatives were associated with the blockage of allergen-mediated immunoglobulin E hypersensitivity, T-cell-mediated cytolysis, formation of migration inhibitory factor, activation of phagocytic cell function, and release of histamine, slow-reacting substances, and prostaglandins (38, 41–43). Furthermore, induced chronic arthritis was related to decreased cyclic adenosine monophosphate levels, increased lysosomal enzymatic activity, and release in the paw (44).

Helenalin, the most potent sesquiterpene lactone anti-inflammatory agent, as well as eupahyssopin and deoxyelephantopin, was observed to elevate the cyclic adenosine monophosphate level of mouse and rat liver significantly after *in vivo* administration whereas tenulin had little effect. The pseudoguaianolide sesquiterpene lactones, except the isotenulin series, significantly elevated the cyclic adenosine monophosphate levels of polymorphonuclear neutrophils whereas the germacranolide sesquiterpene lactones either had no effect or the opposite effect.

The mode of action of sesquiterpene lactones as anti-inflammatory agents appeared to be similar to commercially available agents. These agents were shown to be potent inhibitors of neutrophil migration, lysosomal rupture and enzymatic activity, and prostaglandin synthesis, which was linked to elevated cyclic adenosine monophosphate levels.

REFERENCES

(1) I. H. Hall, K. H. Lee, C. O. Starnes, Y. Sumida, R. Y. Wu, T. G. Waddell, J. W. Cochran, and K. G. Gerhart, *J. Pharm. Sci.*, **68**, 537 (1979).

(2) K. H. Lee and T. A. Geissman, *Phytochemistry*, **9**, 403 (1970).

(3) K. H. Lee, D. C. Anuforo, E. S. Huang, and C. Piantadosi, *J. Pharm. Sci.*, **61**, 626 (1972).

(4) I. H. Hall, K. H. Lee, E. C. Mar, C. O. Starnes, and T. G. Waddell, *J. Med. Chem.*, **20**, 333 (1971).

(5) R. A. Lucas, S. Rovinski, R. J. Kiesel, L. Dortman, and H. B. Mac Phillamy, *J. Org. Chem.*, **29**, 1549 (1964).
(6) K. H. Lee, T. Kimura, M. Haruna, A. T. McPhail, K. D. Onan, and H. C. Huang, *Phytochemistry*, **16**, 1068 (1977).
(7) K. H. Lee, T. Kimura, M. Okamoto, C. M. Cowherd, A. T. McPhail, and K. D. Onan, *Tetrahedron Lett.*, **14**, 1051 (1976).
(8) K. H. Lee, T. Ibuka, H. C. Huang, and D. L. Harris, *J. Pharm. Sci.*, **64**, 1077 (1975).
(9) K. H. Lee, H. Furukawa, M. Kozuka, H. C. Huang, P. A. Luchan, and A. T. McPhail, *J. Chem. Soc., Chem. Commun.*, **1973**, 476.
(10) A. T. McPhail, K. D. Onan, K. H. Lee, T. Ibuka, M. Kozuka, T. Shingu, and H. C. Huang, *Tetrahedron Lett.*, **1974**, 2739.
(11) K. H. Lee, C. M. Cowherd, and M. T. Walo, *J. Pharm. Sci.*, **64**, 1572 (1975).
(12) K. H. Lee, H. C. Huang, E. S. Huang, and H. Furukawa, *ibid.*, **61**, 629 (1972).
(13) K. H. Lee, T. Ibuka, A. T. McPhail, K. D. Onan, T. A. Geissman, and T. G. Waddell, *Tetrahedron Lett.*, **1974**, 1149.
(14) T. G. Waddell, A. M. Austin, J. W. Cochran, K. G. Gerhart, I. H. Hall, and K. H. Lee, *J. Pharm. Sci.*, **68**, 715 (1979).
(15) G. M. Ksander, J. E. McMurry, and M. Johnson, *J. Org. Chem.*, **42**, 1180 (1977).
(16) A. P. Roszkowski, W. H. Rooks, A. Tomolonis, and L. M. Miller, *J. Pharmacol. Exp. Ther.*, **179**, 114 (1971).
(17) C. A. Winter, E. A. Risley, and G. W. Nuss, *Proc. Soc. Exp. Biol. Med.*, **111**, 544 (1962).
(18) B. H. Waksman, C. M. Pearson, and J. T. Sharp, *J. Immunol.*, **85**, 403 (1960).
(19) D. English and B. R. Anderson, *J. Immunol. Methods*, **5**, 249 (1974).
(20) R. P. Orange, M. D. Valentine, and K. F. Austen, *J. Exp. Med.*, **127**, 767 (1968).
(21) I. H. Hall and G. L. Carlson, *J. Med. Chem.*, **19**, 1257 (1976).
(22) R. Gianetto and C. deDuve, *Biochem. J.*, **59**, 433 (1955).
(23) P. S. Chen, T. Y. Toribara, and H. Warner, *Anal. Chem.*, **28**, 1756 (1956).
(24) W. D. Schleuning and H. Fritz, *Methods Enzymol.*, **XLV**, 330 (1976).
(25) R. J. Smith, C. Sabin, H. Gilchrest, and S. Williams, *Biochem. Pharmacol.*, **25**, 2171 (1976).
(26) A. L. Steiner, D. M. Kipnis, R. Utiger, and C. Parker, *Proc. Natl. Acad. Sci. USA*, **64**, 367 (1969).
(27) R. V. Tomlinson, H. J. Ringold, M. C. Qureshi, and E. Forchielli, *Biochem. Biophys. Res. Commun.*, **46**, 552 (1972).
(28) M. Glatt, H. Kälin, K. Watner, and K. Borene, *Agents Action*, **7**, 321 (1977).
(29) R. D. Nelson, P. G. Quie, and R. L. Simmons, *J. Immunol.*, **115**, 1650 (1975).
(30) C. Deby, Z. M. Bacq, and P. Van Cuneghem, in "Perspectives in Inflammation;" D. A. Willoughby, J. P. Givoud, and G. P. Velo, Eds., University Park Press, Baltimore, Md., 1977, p. 339.
(31) M. W. Whitehouse and P. D. G. Dean, *Biochem. Pharmacol.*, **14**, 557 (1965).
(32) M. W. Whitehouse and J. M. Haslam, *Nature*, **196**, 1323 (1962).
(33) R. E. Bryant, R. M. de Prez, M. H. Van Way, and D. E. Roger, *J. Exp. Med.*, **124**, 483 (1966).
(34) P. C. Wilkinson, "Chemotaxis and Inflammation," Churchill and Livingstone, London, England, 1974, pp. 62, 63.
(35) O. Rojas-Espinosa and A. M. Dannenberg, in "Inflammation and Anti-inflammatory Therapy," G. Katona and J. R. Blengio, Eds., Spectrum, New York, N.Y., 1974, p. 31.
(36) M. A. Verity and W. J. Brown, *Biochim. Biophys. Acta*, **151**, 289 (1968).
(37) E. M. Press, R. R. Porter, and J. Cebra, *Biochem. J.*, **74**, 501 (1960).
(38) E. Arrigoni-Martelli, "Inflammation and Anti-inflammatories," Spectrum, New York, N.Y., 1977, pp. 85–98.
(39) I. Rivikin and E. L. Becker, *Fed. Proc. Fed. Am. Soc. Exp. Biol.*, **31**, 657 (1972).
(40) R. L. Tse, P. Phelps, and D. Urban, *J. Lab. Clin. Med.*, **80**, 264 (1972).
(41) H. R. Borene, L. M. Lichtenstein, K. L. Melmon, C. S. Henney, Y. Weinstein, and G. M. Shearer, *Science*, **184**, 19 (1974).
(42) M. Kalimer and K. F. Austin, *Biochem. Pharmacol.*, **23**, 763 (1974).
(43) C. W. Parker, T. J. Sullivan, and H. J. Wedner, *Adv. Cycl. Nucleotides*, **4**, 1 (1974).
(44) M. J. Parnhem, I. L. Bonta, and M. J. P. Adolfs, in "Perspectives in Inflammation," D. A. Willoughby, J. P. Giroud, and G. P. Velo, Eds., University Park Press, Baltimore, Md., 1977, pp. 279–287.

ACKNOWLEDGMENTS

Supported by University of North Carolina Research Council Grant VF 475 (awarded to I. H. Hall); National Institutes of Health Grant CA-17625 and American Cancer Society Grants CH-19A, CH-19B, and CH-19C (awarded to K. H. Lee); and a Research Corporation of America grant and an American Chemical Society grant (awarded to T. G. Waddell).