

- (20) R. J. Baldessarini, A. E. Boyd, III, N. S. Kula, and R. J. Borgman, *Psychoneuroendocrinology*, **4**, 173 (1979).
- (21) P. N. Kaul, E. Brockmann-Hanssen, and E. L. Way, *J. Pharm. Sci.*, **50**, 248 (1961).
- (22) A. M. Burkman, R. E. Notari, and W. K. Van Tyle, *J. Pharm. Pharmacol.*, **26**, 493 (1974).
- (23) R. V. Smith, R. E. Wilcox, W. H. Soine, W. H. Riffée, R. J. Baldessarini, and N. S. Kula, *Res. Commun. Chem. Pathol. Pharmacol.*, **24**, 483 (1979).
- (24) P. Protais, J. Costentin, and J. C. Schwartz, *Psychopharmacology*, **50**, 1 (1976).
- (25) W. H. Riffée, R. E. Wilcox, and R. V. Smith, *Eur. J. Pharmacol.*, **54**, 723 (1979).
- (26) R. E. Wilcox, W. H. Riffée, and R. V. Smith, *Pharmacol. Biochem. Behav.*, **10**, 161 (1979).
- (27) R. E. Wilcox, W. H. Riffée, and R. V. Smith, *ibid.*, **11**, 653 (1979).
- (28) W. H. Soine, P. Salgo, and R. V. Smith, *J. Labelled Compd. Radiopharm.*, **16**, 597 (1979).
- (29) R. V. Smith, D. W. Humphrey, S. Szeinbach, and J. C. Glade, *Anal. Lett.*, **12**, 371 (1979).
- (30) R. V. Smith, A. E. Klein, A. M. Clark, and D. W. Humphrey, *J. Chromatogr.*, **179**, 195 (1979).
- (31) R. V. Smith, A. E. Klein, and D. O. Thompson, *Mikrochim. Acta*, **151** (1980) I.
- (32) B. H. Migdalof, *Drug Metab. Rev.*, **5**, 295 (1976).
- (33) R. V. Smith, D. W. Humphrey, and R. E. Wilcox, *Res. Commun. Chem. Pathol. Pharmacol.*, **27**, 183 (1980).
- (34) J. W. Crow, M. Gibaldi, and G. Levy, *J. Pharm. Sci.*, **68**, 958 (1979).
- (35) P. N. Kaul and M. W. Conway, *ibid.*, **60**, 93 (1971).
- (36) R. V. Smith, J. C. Glade, and D. W. Humphrey, *J. Chromatogr.*, **172**, 520 (1979).
- (37) J. G. Cannon, R. V. Smith, A. Modiri, S. P. Sood, R. J. Borgman, M. A. Aleem, and J. P. Long, *J. Med. Chem.*, **15**, 273 (1972).
- (38) G. M. McKenzie and H. L. White, *Biochem. Pharmacol.*, **22**, 2329 (1973).
- (39) K. Missala, S. Lal, and T. L. Sourkes, *Eur. J. Pharmacol.*, **22**, 54 (1973).
- (40) A. L. Symes, S. Lal, and T. L. Sourkes, *J. Pharm. Pharmacol.*, **27**, 947 (1975).
- (41) G. Levy and M. Gibaldi, in "Handbook of Experimental Pharmacology," Vol. XXVIII Part 3, J. R. Gillette and J. R. Mitchell, Eds., Springer-Verlag, New York, N.Y., 1974, pp. 1-34.

ACKNOWLEDGMENTS

Supported by Grants NS-12259 (R. V. Smith) and NS-06114 (R. E. Wilcox) from the National Institute for Neurological and Communicative Disorders and Stroke and Grant MH-33442 (W. H. Riffée and R. E. Wilcox) from the National Institute of Mental Health. Assistance was also obtained through a starter grant (R. E. Wilcox) from the University Research Institute (BRSG-Program).

The authors are grateful to Julie A. Anderson, Astreida Ducreux-P., and Dawn T. Sanchez-Barona for technical assistance and to the Sterling Winthrop Research Institute, Rensselaer, N.Y., for their generous gift of *N-n*-propylmorphine.

Antitumor Agents XLII: Comparison of Antileukemic Activity of Helenalin, Brusatol, and Bruceantin and Their Esters on Different Strains of P-388 Lymphocytic Leukemic Cells

IRIS H. HALL*, K. H. LEE, M. OKANO, D. SIMS, T. IBUKA, Y. F. LIOU, and Y. IMAKURA

Received September 8, 1980, from the Division of Medicinal Chemistry, School of Pharmacy, University of North Carolina, Chapel Hill, NC 27514. Accepted for publication March 6, 1981.

Abstract □ Based on the fact that some known antineoplastic agents possess an ester moiety within their structure, the esters of helenalin, a sesquiterpene lactone, and of brusatol and bruceantin, quassinoids, were synthesized and tested for antileukemic activity in the P-388 screen. These agents gave different T/C % values dependent on the P-388 lymphocytic leukemia strain and the host strain of mice used. Later studies demonstrated that the agents caused different degrees of inhibition of nucleic acid and protein synthesis in the various P-388 strains. The higher the degree of inhibition of precursor incorporation into the nucleic acid or protein, the higher was the T/C % value obtained in a given P-388 strain. The study demonstrates the lack of consistency of P-388 lymphocytic leukemia cell lines used in various laboratories and indicates

that the inbred strain of mice is a critical factor in the tolerance of drug toxicity and, thus, T/C % obtained.

Keyphrases □ P-388 lymphocytic leukemic cells—antileukemic activity of helenalin, brusatol, and bruceantin and their esters □ Antileukemic agents—comparison of helenalin, brusatol, and bruceantin and their esters □ Helenalin—comparison with brusatol, bruceantin, and their esters, antileukemic activity □ Brusatol—comparison with helenalin and bruceantin and their esters, antileukemic activity □ Bruceantin—comparison with helenalin and brusatol and their esters, antileukemic activity

The naturally occurring sesquiterpene lactones continue to provide numerous examples of structures exhibiting significant cytotoxic antitumor activity (1). In general, they have proven to be potent inhibitors of Walker 256 carcinosarcoma growth in rats and Ehrlich ascites carcinoma growth in mice and marginal inhibitors of P-388 lymphocytic leukemia in mice (2). For example, eupaformosanin (3), molephantinin (4), phantomolin (5), eupahyssopin (6),

and helenalin (I) (7) all demonstrated highly significant inhibitory activity in the Walker 256 carcinosarcoma survival system with a T/C value of $\geq 300\%$ at the low dose of 2.5 mg/kg (T/C $> 140\%$ required for significant activity). However, in the P-388 murine lymphocytic leukemia screen, an *in vivo* test system currently used as a standard method by the National Cancer Institute (NCI) for evaluating compounds of natural origin, these compounds

Table I—Antineoplastic Activity of Sesquiterpene Lactones and Quassinoids against P-388-UNC Lymphocytic Leukemia Cell Growth in BDF₁ Mice

Compound	Dose, mg/kg/day ip	Average Days Survived of T/C	T/C %
I	60 ^a	3.0/9.66	31
	15	11.9/9.66	123
	8	15.4/9.66	162
	3	12.9/9.66	134
II	60 ^a	5.8/9.66	60
	30	16.2/9.66	168
	15	25.2/9.66	261
	8	13.8/9.66	143
III	0.6	14.2/9.5	149
	0.3	14.3/9.5	150
	0.25	14.5/9.5	153
	0.125	15.0/9.5	158
IV	0.100	12.8/9.5	134
	1.0	20.2/9.5	213
	0.6	25.8/9.5	272 (197)
	0.3	20.4/9.5	215
V	0.25	15.5/9.5	163
	0.125	11.0/9.5	116
	0.100	11.0/9.5	116
	0.6	25.2	217

^a Toxic at this dose.

failed to give T/C values higher than 147% at the maximum dose level of 25 mg/kg/day (1) (T/C >125% required for significant activity).

BACKGROUND

In this laboratory, previous systematic investigation of the structure-activity relationships among the sesquiterpene lactones and related compounds indicated the importance of the bifunctional alkylating enone $O=C-C=CH_2$ system and the ester moiety for enhanced cytotoxic antitumor activity (1). For example, the bifunctional alkylating helenalin (I), which contains a β -unsubstituted cyclopentenone and an α -methylene- γ -lactone, is more potent than the corresponding 2,3-dihydrohelenalin or 11,13-dihydrohelenalin (plenolin) in its *in vitro* cytotoxicity (8) and *in vivo* antitumor activity (2). Cytotoxicity is also enhanced by introduction of a lipophilic ester side chain to helenalin and related derivatives (9).

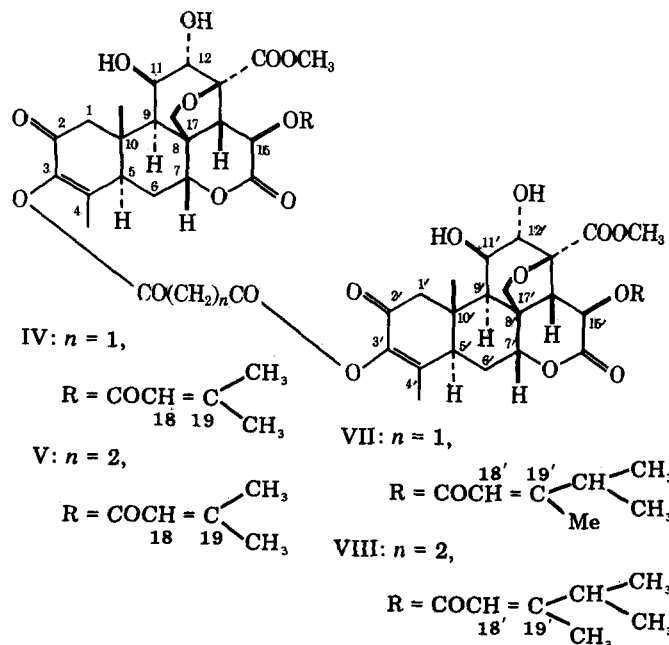
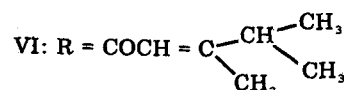
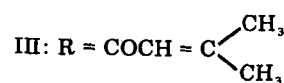
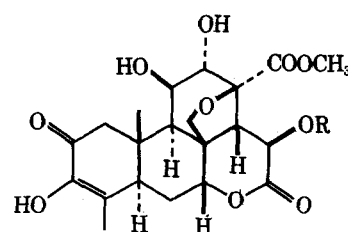
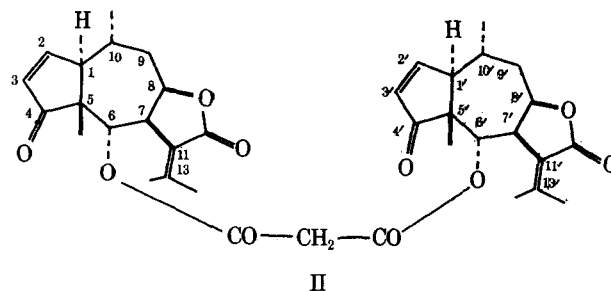
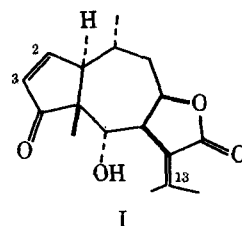
The importance of the ester group that contributes to the enhanced *in vivo* antitumor activity is also seen in many other naturally occurring antitumor agents including the germacranolides eupaformosanin, molephantinin, phantomolin, and eupahysosopin as well as the quassinoid bruceantin (10), the diterpene taxol (11), the alkaloids indicine *N*-oxide (12), homoharringtonine (13), vincristine (14), and vinblastine (14), the macrolide maytansine (15), and the fungal metabolites anguidin (16), actinomycin D (dactinomycin) (17), and mitomycin C (18). The latter compounds (19) possess high *in vivo* antileukemic activity against the P-388 lymphocytic leukemia growth.

This evidence, coupled with the fact that polyfunctionality is a common structural feature in many of the described antileukemic agents, led to the hypothesis that a polyfunctional alkylating-type compound originating from the combination of bifunctional or monofunctional alkylating active species *via* an ester linkage might be highly active as an antileukemic agent. Thus, esters of helenalin, brusatol, and bruceantin were synthesized and examined for their antileukemic activity against P-388 lymphocytic leukemia cell growth. Those results are now reported utilizing two different strains of P-388 lymphocytic leukemia cells along with the effects of these esters on key enzymes of nucleic acid and protein synthesis.

EXPERIMENTAL

Source of Compounds—Helenalin (I) was isolated from *Balduina angustifolia* by the methods of Lee *et al.* (20). Treatment of I with malonyl dichloride in dry benzene by the general method gave rise to crys-

¹ The UV, IR, and NMR spectra data of II, IV, V, VII, and VIII were in accord with assigned structure.



talline bis(helenalinyl)malonate¹ [II, $C_{33}H_{36}O_{10}$, mp 217–218°], resulting in a molecule with four alkylating centers. Brusatol (III) was obtained by treating bruceoside A, a quassinoid glycoside isolated from *Brucea javanica*, with 3 *N* H₂SO₄-methanol (1:1) to hydrolyze the glycosidic

Table II—Antineoplastic Activity of Sesquiterpene Lactones and Quassinoids against P-388-UNC and P-388-NIH Lymphocytic Leukemia Cell Growth

Compound	Dose, mg/kg/day ip	P-388-UNC, T/C %		P-388-NIH, T/C %	
		CDF	BDF ₁	CDF	BDF ₁
I	15	123	—	134	—
	8	168	—	154	162
	3	134	158	138	152
II	15	—	—	toxic	toxic
	8	145	168	129	145
	3	143	—	138	130
III	1.0	152 (toxic)	234	162 (toxic)	176
	0.6	149	152	152	156
	0.6	188	197	125	135
IV	0.6	168	207	133	161
V	1.0	168	258	162	176
VI	0.6	146	—	—	—
VII ^a	1.2	—	111	—	—
	0.6	138	139	125	139
	0.3	—	109	—	—
VIII	1.2	—	164	—	168
	0.6	132	153	144	151
	0.3	—	150	—	—
5-Fluorouracil	12.5	162	166	186	209

^a P-388-NIH contractors: 163 (3.0), 165 (1.5), 146 (0.75), and 128 (0.38).

Table III—*In Vitro* Effects (Percent of Control) of Sesquiterpene Lactones and Quassinoids on P-388-UNC and P-388-NIH Lymphocytic Leukemia Cell Metabolism

Compounds	P-388-UNC			P-388-NIH		
	DNA	RNA	Protein	DNA	RNA	Protein
I, 20 μ moles	15 $\pm$ 2 ^a	85 $\pm$ 3 ^a	68 $\pm$ 9 ^a	10 $\pm$ 5 ^a	84 $\pm$ 6 ^a	79 $\pm$ 3 ^a
II, 20 μ moles	38 $\pm$ 3 ^a	55 $\pm$ 4 ^a	50 $\pm$ 4 ^a	38 $\pm$ 4 ^a	52 $\pm$ 5 ^a	75 $\pm$ 10 ^b
III, 10 μ moles	48 $\pm$ 4 ^a	44 $\pm$ 2 ^a	45 $\pm$ 3 ^a	47 $\pm$ 4 ^a	71 $\pm$ 5 ^a	80 $\pm$ 6 ^b
IV, 10 μ moles	54 $\pm$ 7 ^a	52 $\pm$ 5 ^a	17 $\pm$ 13 ^a	46 $\pm$ 5 ^a	90 $\pm$ 7 ^a	65 $\pm$ 5 ^a
V, 10 μ moles	36 $\pm$ 4 ^a	41 $\pm$ 6 ^a	26 $\pm$ 4 ^a	41 $\pm$ 3 ^a	71 $\pm$ 5 ^a	75 $\pm$ 3 ^a
VI, 10 μ moles	38 $\pm$ 2 ^a	47 $\pm$ 6 ^a	58 $\pm$ 3 ^a	35 $\pm$ 3 ^a	71 $\pm$ 3 ^a	85 $\pm$ 2 ^b
VII, 10 μ moles	45 $\pm$ 8 ^a	66 $\pm$ 3 ^a	60 $\pm$ 3 ^a	43 $\pm$ 4 ^a	67 $\pm$ 4 ^a	75 $\pm$ 4 ^a
VIII, 10 μ moles	52 $\pm$ 4 ^a	78 $\pm$ 4 ^a	68 $\pm$ 3 ^a	52 $\pm$ 5 ^a	74 $\pm$ 6 ^a	77 $\pm$ 2 ^a
0.05% Polysorbate 80 (control)	100 $\pm$ 6	100 $\pm$ 5	100 $\pm$ 4	100 $\pm$ 7	100 $\pm$ 5	100 $\pm$ 8
Emetine, 50 μ moles	—	—	60 $\pm$ 3 ^a	—	—	61 $\pm$ 4 ^a
Pyrocatechol violet, 50 μ moles	—	—	60 $\pm$ 5 ^a	—	—	62 $\pm$ 4 ^a
Value dpm/10 ⁶ cells (<i>n</i> = 6)	36,777	38,615	76,518	39,564	45,309	79,103

^a *p* $\leq$ 0.001. ^b *p* $\leq$ 0.005.

linkage (21–23). Bruceantin (VI) was also synthesized from bruceoside A (24–25).

Brusatol (III) (22–24) and bruceantin (VI) were treated similarly with malonyl dichloride and succinyl chloride in dry pyridine to yield, after purification of the reaction products by preparative TLC, the corresponding bis(brusatolyl)malonate¹ [IV, C₅₅H₆₄O₂₄·H₂O, mp 191–193°], bis(brusatolyl)succinate¹ [V, C₅₆H₆₆O₂₄, mp 248–250°], bis(bruceantiny)malonate¹ [VII, C₅₉H₇₂O₂₄, mp 193–195°], and bis(bruceantiny)succinate¹ [VIII, C₆₀H₇₄O₂₄, mp 250° dec.]².

Biological—P-388 Lymphocytic Leukemia Antitumor Screen—The P-388 lymphocytic leukemia-UNC tumor line was originally obtained from Mason Research Institute through NCI and has been maintained in this laboratory in DBA/2 male donor mice for ~2 years. The P-388 lymphocytic leukemia-NIH was obtained in April 1980 from the same source. The eight test compounds with the standard 5-fluorouracil were administered to both the P-388-UNC and P-388-NIH strains as follows. BDF₁ or CDF male mice (~20 g) were administered 10⁶ P-388 lymphocytic leukemia cells intraperitoneally on Day 0 (26). Test compounds were homogenized in 0.05% polysorbate 80–water and administered intraperitoneally daily.

KB Tissue Cytotoxic Assay—KB (human epidermoid carcinoma of the mouth) cells were maintained in minimum essential medium and 10% fetal calf serum containing the antibiotics penicillin and streptomycin (26). The method of Huang *et al.* (27) was utilized for the cytotoxic assay in microtest tissue culture plates.

P-388 Lymphocytic Leukemia Cell Metabolism—BDF₁ male mice (~22 g) were inoculated with 10⁶ P-388 tumor cells as previously described. On day 9, the cells were collected from the peritoneal cavity. *In vitro* incorporation studies were conducted using 10⁶ P-388-UNC or P-388-NIH cells, minimum essential medium, and 1 μ Ci of thymidine (6-³H, 21.8 Ci/mmmole), uridine (6-³H, 22.4 Ci/mmmole), or L-leucine [4,5-³H(N) 56.5 Ci/mmmole] in a total volume of 1 ml, which was incubated

for 90 min at 37° (28). Compounds I and II were tested at a final concentration of 20 μ moles, and III–VIII were tested at a 10- μ mole concentration.

The thymidine incorporation into DNA assays were terminated with 0.5 *N* perchloric acid containing 1% pyrophosphate. Radiolabeled DNA was collected on glass fiber paper (GF/F) by vacuum suction. RNA and protein incorporation studies were terminated with trichloroacetic acid, and the acid-insoluble macromolecule was collected on nitrocellulose membrane filters. The filter papers were placed in scintillation vials and counted in a toluene–octoxynol scintillation fluid (29).

RESULTS AND DISCUSSION

Initially, the antileukemic activity was determined in the P-388-UNC lymphocytic leukemia screen. The T/C % value for II at 15 mg/kg/day was exceedingly high, *e.g.*, T/C % = 261 in BDF₁ male mice. The quassinoid esters of III were then examined and, as can be seen in Table I, IV at 0.6 mg/kg resulted in T/C % values of 272 and 197 and V resulted in a T/C % value of 217 in BDF₁ male mice.

Compounds I–V followed a dose–response curve against P-388-UNC lymphocytic leukemia growth in BDF₁ mice. The ED₅₀ for helenalin (I) in the KB cytotoxic assay was 0.188 compared to the literature value (28) of 0.19 μ g/ml. Bis(helenaliny)malonate (II) afforded an ED₅₀ of 0.08 μ g/ml. The ED₅₀ values for the quassinoids were also impressive, with III, IV, VI, and VIII equal to 0.31, 0.025, 0.056, and 0.275 μ g/ml, respectively.

In an attempt to confirm these unexpected high results, II, IV, and V were submitted to NCI for retesting and evaluation. The two contract screening agencies used by NCI were unable to obtain results >12 mg/kg in CDF mice for I and II; but at 3 and 0.39 mg/kg, T/C % values of 130³ and 120⁴, respectively, were obtained. Compound IV was also submitted to the same two contract screeners³, whose studies resulted in T/C %

² Unpublished results.

³ Southern Research.

values of 131, 135, and 131 at 3, 1.5, and 0.75 mg/kg, respectively. The second contract screener⁴ found II, III, and IV totally inactive in their P-388 lymphocytic leukemia screen in CDF mice.

Since these results were somewhat baffling, a new sample of P-388 lymphocytic leukemia cells, noted as P-388-NIH, was obtained⁵. The compounds were retested against P-388-UNC and P-388-NIH leukemia growth in CDF and BDF₁ male mice with dosing from Days 1 to 9. These results are shown in Table II along with the data for two new compounds, i.e., the malonate (VII) and succinate (VIII) ester of bruceantin. Bruceantin (VI) is a quassinoid in Phase II clinical trial. These data confirmed previous observation by this laboratory in BDF₁ male mice using the P-388-UNC strain, whereas data for P-388-NIH in CDF mice were comparable with the contractor's results. However, a difference could be observed between BDF₁ and CDF donor mice and the resulting T/C % values. Apparently, the BDF₁ male mice were able to tolerate the higher doses of I-III and VI, thus affording higher T/C % values than when administered to CDF mice. In fact, in a previous report (29) on DBA/2 male mice, testing above 0.5 mg/kg with III in the P-388 screen resulted in deaths. The inbred hybrid strain of mice may be a critical factor since mice used for the study in Table I were bred in this laboratory (C₅₇B1/6 female × DBA/2j male), whereas those used in Table II were obtained commercially⁶. When VII was tested³ at 3, 1.5, 0.75, and 0.38 mg/kg, T/C % values of 163, 165, 146, and 128, respectively, were obtained, which were within the scope of data presented in Table II.

The two sets of conflicting data pose the question of whether P-388-UNC is a different strain of P-388 lymphocytic leukemia cells resulting from some alteration or mutation. Thus, since the sesquiterpene lactones and quassinoids were known to affect nucleic acid and protein synthesis in tumor cells, these parameters were examined in the P-388-UNC and P-388-NIH strains of cells from BDF₁ mice.

Table III indicates that *in vitro* DNA synthesis was affected approximately the same degree by I and II in the two strains of P-388 cells. RNA synthesis was inhibited by I and II equally in the P-388-UNC and P-388-NIH cells. Protein synthesis, however, was inhibited at a much higher rate in the P-388-UNC strain than the P-388-NIH strain (e.g., compare II at 50 and 25% inhibition, respectively). Examination of II-VIII showed that the degree of inhibition of DNA synthesis was of the same magnitude in both strains of P-388 cells for a given compound. The RNA synthesis inhibition by III-VI was much higher in the P-388-UNC strain compared to the P-388-NIH strain, resulting in ~24-38% higher inhibition of RNA synthesis, with IV demonstrating the largest difference (i.e., 48% in the P-388-UNC strain and 10% in the P-388-NIH strain).

Protein synthesis inhibition was also decidedly different between the two different strains for III-VI. Compound III afforded a 20% inhibition in the P-388-NIH and a 55% inhibition in the P-388-UNC strain. Compound IV demonstrated a difference of 35% compared to 83% inhibition, V showed a difference of 25% compared to 72%, and VI showed a difference of 15% compared to 42% in the respective strains. Compounds VI and VIII demonstrated approximately the same T/C % values in the two strains of P-388 cells, and the effects of the two compounds on RNA and protein synthesis was comparable in the two strains of cells. The standard emetine and pyrocatechol violet at 5 μ moles demonstrated the same degrees of protein inhibition in the two strains of P-388 cells.

These studies indicate that the P-388-UNC and P-388-NIH strains of P-388 lymphocytic leukemia cells are different in their metabolic response to certain chemical agents and the difference may be responsible for diverse values obtained in the antineoplastic screen. The P-388-UNC strain appears to be particularly sensitive to sesquiterpene lactone and quassinoid-type structure but less sensitive to 5-fluorouracil. Furthermore, the strain of mice used may be critical for the T/C % values obtained, depending on the tolerance of the mice to the particular toxic effects of the chemical agent.

REFERENCES

- (1) K. H. Lee, "Program and Abstracts of the 16th National Medic-

⁴ Battelle Memorial Institute.

⁵ Mason Research Institute.

⁶ Jackson Laboratories.

inal Chemistry Symposium," American Chemical Society, Kalamazoo, Mich. 1978, pp. 44-58.

(2) I. H. Hall, K. H. Lee, C. O. Starnes, S. ElGebaly, T. Ibuka, Y. S. Wu, T. Kimura, and M. Haruna, *J. Pharm. Sci.*, **67**, 1235 (1978).

(3) K. H. Lee, T. Kimura, M. Haruna, A. T. McPhail, K. D. Onan, and H. C. Huang, *Phytochemistry*, **16**, 1068 (1977).

(4) K. H. Lee, T. Ibuka, H. C. Huang, and D. L. Harris, *J. Pharm. Sci.*, **64**, 1077 (1975).

(5) A. T. McPhail, K. D. Onan, K. H. Lee, T. Ibuka, M. Kozuka, T. Shingu, and H. C. Huang, *Tetrahedron Lett.*, **1974**, 2739.

(6) K. H. Lee, T. Kimura, M. Okamoto, C. M. Cowherd, A. T. McPhail, and K. D. Onan, *ibid.*, **1976**, 1051.

(7) K. H. Lee, M. Haruna, H. C. Huang, B. S. Wu, and I. H. Hall, *J. Pharm. Sci.*, **66**, 1194 (1977).

(8) K. H. Lee, H. Furukawa, and E. S. Huang, *J. Med. Chem.*, **15**, 609 (1972).

(9) K. H. Lee, R. Meck, C. Piantadosi, and E. S. Huang, *ibid.*, **16**, 299 (1973).

(10) S. M. Kupchan, R. W. Britton, J. A. Lacadie, M. F. Ziegler, and C. W. Sigel, *J. Org. Chem.*, **40**, 648 (1975).

(11) M. C. Wani, H. L. Taylor, M. E. Wall, P. Coggon, and A. T. McPhail, *J. Am. Chem. Soc.*, **93**, 2325 (1971).

(12) A. R. Mattocks, *J. Chem. Soc. (C)*, **1967**, 329.

(13) R. G. Powell, D. Weisleder, and C. R. Smith, Jr., *J. Pharm. Sci.*, **61**, 1227 (1972).

(14) I. S. Johnson, J. G. Armstrong, M. Gorman, and J. P. Burnett, *Cancer Res.*, **23**, 1390 (1963).

(15) S. M. Kupchan, Y. Komoda, W. A. Court, G. J. Thomas, R. M. Smith, A. Karim, C. J. Gilmore, R. C. Haltiwanger, and R. F. Bryan, *J. Am. Chem. Soc.*, **94**, 1354 (1972).

(16) W. Loeffler, R. Mauli, M. E. Ruesch, and H. Staehelin, *Chem. Abstr.*, **62**, 5856d (1965).

(17) A. Goldin and R. K. Johnson, *Cancer Chemother. Rep. Pt. 1*, **58**, 63 (1974).

(18) C. L. Stevens, K. G. Taylor, M. E. Munk, W. S. Marshall, K. Noll, G. D. Shah, L. G. Shah, and K. Uzu, *J. Med. Chem.*, **8**, 1 (1964).

(19) H. B. Wood, Jr., "Chemical Structures of Current Interest to the Division of Cancer Treatment—Drugs with Clinical Activity and Some Unique Compounds in Development," Drug Synthesis and Chemistry Branch, National Cancer Institute, Jan. 1977.

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

(21) K. Y. Sim, J. J. Sims, and T. A. Geissman, *J. Org. Chem.*, **33**, 429 (1968).

(22) K. H. Lee, Y. Imakura, and H. C. Huang, *J. Chem. Soc., Chem. Commun.*, **1977**, 69.

(23) K. H. Lee, Y. Imakura, Y. Sumida, R. Y. Wu, I. H. Hall, and H. C. Huang, *J. Org. Chem.*, **44**, 2180 (1979).

(24) S. M. Kupchan, R. W. Britton, J. A. Lacadie, M. F. Ziegler, and C. W. Sigel, *ibid.*, **40**, 648 (1975).

(25) M. Okano and K. H. Lee, *J. Org. Chem.*, in press.

(26) R. I. Geran, N. H. Greenberg, M. M. MacDonald, A. M. Schumacher, and B. J. Abbott, *Cancer Chemother. Rep., Part 3*, **3**, 1 (1972).

(27) E. S. Huang, K. H. Lee, C. Piantadosi, T. A. Geissman, and J. S. Pagano, *J. Pharm. Sci.*, **61**, 1960 (1972).

(28) G. R. Pettit, J. C. Budzinski, G. M. Cragg, P. Brown, and L. D. Johnston, *J. Med. Chem.*, **17**, 1013 (1974).

(29) I. H. Hall, K. H. Lee, S. A. ElGebaly, Y. Imakura, Y. Sumida, and R. Y. Wu, *J. Pharm. Sci.*, **68**, 883 (1979).

ACKNOWLEDGMENTS

Supported by American Cancer Society Grant CH-19 and National Institutes of Health Grants CA-17625 and CA-22929 awarded to K. H. Lee and I. H. Hall.

The authors thank Larry Carpenter and Jill Maness for technical assistance.