Articles

Antitumor Agents. 44.[1] Bis(helenalinyl) Esters and Related Derivatives as Novel Potent Antileukemic Agents

Kuo-Hsiung Lee,* Toshiro Ibuka, Donald Sims, Osamu Muraoka, Hiroshi Kiyokawa, Iris H. Hall,

Department of Medicinal Chemistry, School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina 27514

and Hyeong L. Kim

Department of Veterinary Physiology and Pharmacology, Texas A & M University, College Station, Texas 77843.
Received October 31, 1980

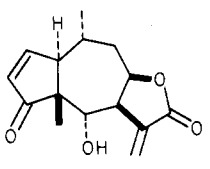
Bis(helenalinyl), bis(plenolinyl), bis(2,3-dihydrohelenalinyl), and bis(2,3,11,13-tetrahydrohelenalinyl) esters have been synthesized in an effort to elucidate the role of the two enone alkylating centers, β-unsubstituted cyclopentenone and α-methylene γ-lactone, as well as the significance of the diester linkage with respect to the enhanced in vivo P-388 lymphocytic leukemia antileukemic activity of bis(helenalinyl) malonate (**2**) against P-388 lymphocytic leukemia in the mouse. The bisesters (**2–5**; **7**, **8**; **10**, **11**) are, in general, more potent and less toxic than their corresponding parent alcohols (**1**, **6**; **9**; **14**). The β-unsubstituted cyclopentenone ring and the α-methylene γ-lactone moiety in the bisesters play important roles for the enhancement of the P-388 antileukemic activity. Removal of the enone double bonds in both alkylating centers of **2** gave rise to inactive compounds. Except for **2**, the potent antileukemic activity of the bis(helenalinyl) esters (**3–5**) appears to be independent of the ester chain length.

In a previous communication,[2] we demonstrated that a combination of two bifunctional alkylating sesquiterpene lactones, such as two helenalins (**1**), through a diester linkage gave rise to greatly enhanced antileukemic activity in P-388 lymphocytic leukemia (maintained in this laboratory in BDF_1 male mice) with decreased toxicity. For example, bis(helenalinyl) malonate (**2**) showed a T/C of 261% at 15 mg/kg with a loss of the toxicity when compared to **1** at the same dose level (see Table I). As an extension to these important findings, we performed a further detailed investigation on the structure–antileukemic activity relationships among the bis(helenalinyl) malonate related derivatives. As also indicated in our previous systematic studies,[3] an enone O=CC=CH_2 system, either present in the form of a β-unsubstituted cyclopentenone or an α-methylene γ-lactone, as seen in **1**, is regarded as an alkylating center and is directly responsible for its cytotoxic antitumor activity, possibly via a rapid Michael-type addition of the biological nucleophiles of key regulatory enzymes of nucleic acid and chromatin metabolism.[4,5] Thus, **2** possesses four alkylating centers. Accordingly, it appeared important to elucidate the role of the aforementioned two enone systems and the number of the alkylating centers, as well as the significance of the diester linkage with respect to the increased antileukemic activity observed in **2**. We report herein the synthesis and P-388 antileukemic activity of bis(helenalinyl) related malonates and succinates, as well as bis(helenalinyl) glutarate and adipate.

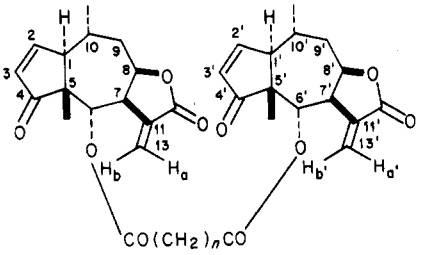
Chemistry. Helenalin (**1**), 11,13-dihydrohelenalin (i.e., plenolin) (**6**), 2,3-dihydrohelenalin (**9**), and 2,3,11,13-tetrahydrohelenalin (**12**) were selected as model compounds for the preparation of their corresponding bisesters (**2–5**; **7**, **8**; **10**, **11**; and **13**, respectively) possessing different types and numbers of alkylating centers. Thus, compounds **2–5** contain four alkylating centers (i.e., two β-unsubstituted cyclopentenones and two α-methylene γ-lactones). Compounds **7** and **8**, and **10** and **11** bear two β-unsubstituted cyclopentenones and two α-methylene γ-lactones, respectively. Compound **13** is devoid of any alkylating center. Compounds **1**, **6**, **9**, and **12** were obtained by methods described previously.[6-8] Plenolin (**6**) was prepared in a better yield (50%) by catalytic reduction of **1** using nickel boride as catalyst. Earlier procedures[8,9] involving catalytic hydrogenation with PtO_2 as catalyst for the conversion of **1** to **6** gave usually less than 20% of yield. Catalytic hydrogenation of **1** led, in addition to the formation of **12**, to a second product (**15**) whose composition, as well as spectral data, indicated the identity of **15** as 2,3-dihydroisohelenalin, a compound obtained previously from the reduction of isohelenalin.[10] The bisesters **2–5**; **7**, **8**; **10**, **11**; and **13** were synthesized by reaction of **1**, **6**, **9**, and **12**, respectively, with the corresponding acid dichlorides in dry benzene under reflux, followed by the usual workup and purification with preparative TLC. The succinate (**3**), glutarate (**4**), and adipate (**5**) of **1** were prepared in order to examine whether the difference in the ester chain length might affect the potential antileukemic

(1) This paper has been presented in part. See "Abstracts of Papers", Second Chemical Congress of the North American Continent, Las Vegas, NV, Aug 24–29, 1980, American Chemical Society, Washington, DC, Abstr MEDI 044. For part 43 see M. Okano and K. H. Lee, *J. Org. Chem.*, **46**, 1138 (1981).
(2) I. H. Hall, K. H. Lee, M. Okano, D. Sims, T. Ibuka, and Y. F. Liou, *J. Pharm. Sci.*, in press.
(3) K. H. Lee, "Program and Abstracts"; 16th National Medicinal Chemistry Symposium of the American Chemical Society, Kalamazoo, MI, 1978, American Chemical Society: Washington, DC, 1978, pp 44–58, and references cited therein.
(4) K. H. Lee, I. H. Hall, E. C. Mar, C. O. Starnes, S. A. ElGebaly, T. G. Waddell, R. I. Hadgraft, C. G. Ruffner, and I. Weidner, *Science*, **196**, 533 (1977).
(5) I. H. Hall, K. H. Lee, E. C. Mar, C. O. Starnes, and T. G. Waddell, *J. Med. Chem.*, **20**, 333 (1977).
(6) K. H. Lee and T. A. Geissman, *Phytochemistry*, **9**, 403 (1970).
(7) K. H. Lee, H. Furukawa, and E. S. Huang, *J. Med. Chem.*, **15**, 609 (1972), and references cited therein.
(8) K. H. Lee, S. H. Kim, H. Furukawa, C. Piantadosi, and E. S. Huang, *J. Med. Chem.*, **18**, 59 (1975).
(9) R. Adams and W. Herz, *J. Am. Chem. Soc.*, **71**, 2554 (1949).
(10) D. Sims, K. H. Lee, R. Y. Wu, H. Furukawa, M. Itoigawa, and Y. Yonaha, *J. Nat. Prod.*, **42**, 282 (1979), and references cited therein.

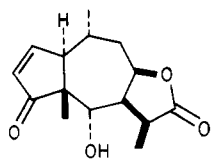
0022-2623/81/1824-0924$01.25/0



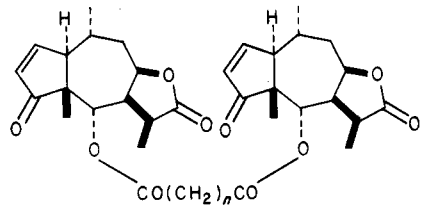
1



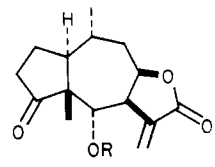
2, n = 1
3, n = 2
4, n = 3
5, n = 4



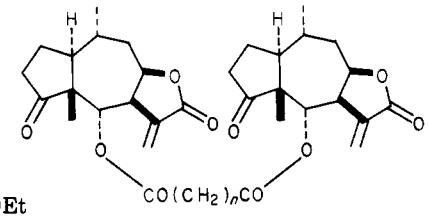
6



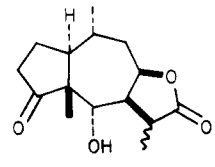
7, n = 1
8, n = 2



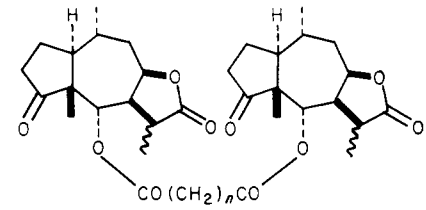
9, R = H
14, R = $COCH_2COOEt$



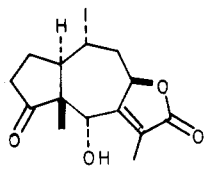
10, n = 1
11, n = 2



12



13



15

activity of the bis(helenalinyl) esters.

Antileukemic Activity and Structure–Activity Relationships. The bisesters prepared in this study were assayed for their in vivo antileukemic activity against P-388 lymphocytic leukemia growth in mice according to the standard National Cancer Institute procedures.[2,11]

Examination of the in vivo data presented in Table I demonstrated (on a milligram per kilogram intraperitoneal dosing regimen) that at 8 (mg/kg)/day compounds 4 and 5 afforded a higher percent T/C value than 1 (percent T/C = 162), whereas at 15 mg/kg compound 2 extended the life expectancy of the mice twofold (percent T/C =261) compared to compound 1 (percent T/C =123). At 25 mg/kg, compound 3 afforded a percent T/C = 178, compared to 1 with a percent T/C = 127. These data would suggest, on a milligram per killogram basis, that the bis(helenalinyl) esters are more potent than the parent alcohol helenalin at specific doses. Examination of bis(phenolinyl) malonate (7) and succinate (8) at 8 mg/kg demonstrated that 7 (percent T/C = 124) and 8 (percent T/C = 145) have higher activity against tumor growth and that they extended the life expectancy compared to 6 with a percent T/C = 111. Compound 8 afforded a percent T/C = 194 at 15 mg/kg compared to plenolin with a percent T/C = 118, again indicating that the bis(plenolinyl) esters were more active than the monomeric plenolin.

Similarly, the bis(2,3-dihydrohelenalinyl) esters (10 and 11) were more potent than their corresponding parent alcohol 9 and its monoester 14 at 15 and 25–30 mg/kg levels (e.g., compare percent T/C = 137 and 141 at 15 mg/kg for 10 and 11 to 114 for 14, respectively, and percent T/C = 157 at 30 mg/kg for 11 to 110 at 25 mg/kg for 9).

The β-unsubstituted cyclopentenone ring plays an important role in activity against P-388 lymphocytic leukemia growth. For example, reduction of the cyclopentenone ring of 1, 2, and 3 to compounds 9, 10, and 11, respectively, results in either the loss or the decrease of activity (compared at 25 mg/kg, 127% of 1 to 110% of 9; at 15 mg/kg, 261% of 2 to 137% of 10; and 178% of 3 at 25 mg/kg to 157% of 11 at 30 mg/kg). Similarly, reduction of the α-methylene grouping of the γ-lactone ring of 1, 2, and 3 to 6, 7, and 8, respectively, gave rise to a marked loss of activity (compare at 8 mg/kg, 162% of 1 to 111% of 6; at 15 mg/kg, 261% of 2 to 112% of 7; and at 8 mg/kg, 168% of 3 to 145% of 8). Further reduction of both cyclopentenone and α-methylene γ-lactone moieties, as in either bis(2,3,11,13-tetrahydrohelenalinyl) malonate (13) or in its corresponding parent alcohol 12, led to a decrease or a loss of activity.

Examination of the in vivo data on the bis(helenalinyl) esters (2–5) on a milligram per kilogram basis indicates that the length of the ester side chain is not critical for antineoplastic activity. However, the malonate ester 2 at 15 mg/kg afforded the best activity with a percent T/C = 261, and the glutarate 4 at 8 mg/kg afforded the next best activity with a percent T/C = 195.

Experimental Section

Chemistry. Unless otherwise specified, melting points were determined on a Thomas-Hoover melting point apparatus and were uncorrected. IR spectra were recorded on a Perkin-Elmer 257 grating spectrophotometer. NMR spectra were measured with a JEOL C-60 HL spectrometer (Me_4Si); chemical shifts are reported in δ (parts per million) units (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet), and the J values are in hertz. Mass spectra were determined on an AEI MS-902 instrument at 70 eV using a direct-inlet system. Silica gel for column chromatography refers to Mallincrodt Silica AR cc-7 (200–325 mesh), silica gel for preparative TLC refers to Merck silica gel GF-254, and silica gel for TLC refers to Merck silica gel G developed with suitable solvent systems [i.e., $CHCl_3$–Me_2CO (3:1), $CHCl_3$–Me_2CO (2:1), $CHCl_3$–Me_2CO (4:1), and $CHCl_3$–Me_2CO (5:1)] and visualized by spraying with 10% sulfuric acid and heating. All new compounds have been rigorously purified to homogeneity by TLC in at least three solvent systems, and their elemental compositions were analyzed by high-resolution mass spectrometry in lieu of combustion, except for 2 and 3, due to the limited supply of the natural helenalin (1).

Helenalin (1) was isolated from the ethanolic extract of Texas *Helenium microcephalum* according to an exact literature procedure.[6]

Plenolin (6) was prepared by catalytic reduction of 1 using nickel boride as a catalyst. The nickel boride catalyst was prepared as follows, according to the literature method.[12] Nickel acetate

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

(12) T. W. Russell and R. C. Hoy, *J. Org. Chem.*, 36, 2018 (1971).

Table I. Antileukemic Activity of Bis(helenalinyl) Esters and Related Compounds against the P-388 Lymphocytic Leukemia in BDF_1 Male Mice (~22 g) Dosed on Days 1-14

compd	dose, (mg/kg)/day, ip	av days survival of treated/control	T/C, %	compd	dose, (mg/kg)/day, ip	av days survival of treated/control	T/C, %
2	60[a]	5.8/9.66	60	10	15	13.2/9.66	137
	30	16.2/9.66	168		8	11.8/9.66	122
	15	25.2/9.66	261				
	8	13.8/9.66	143	11	60	12.6/9.66	130
	3	13.8/9.66	143		30	15.2/9.66	157
					15	13.6/9.66	141
3	50	13.4/9.66	140		4	11.0/9.66	114
	25	17.2/9.66	178		2	11.6/9.66	120
	12.5	16.0/9.66	166				
	8	13.7/9.66	168	13	15	11.1/9.66	115
	4	10.6/9.66	110		5	11.8/9.66	122
	2	11.2/9.66	116	1	60[a]	3.0/9.66	31
4	60	4.7/9.66	48		25	12.3/9.66	127
	30	7.0/9.66	72		15	11.9/9.66	123
	15	9.0/9.66	93		8	15.7/9.66	162
	8	18.8/9.66	195		3	12.9/9.66	134
	4	16.5/9.66	171	6	25	13.3/9.66	138
	2	11.2/9.66	116		15	11.4/9.66	118
					8	10.7/9.66	111
5	60	3.8/9.66	40				
	30	6.7/9.66	69	9	25	10.6/9.66	110
	15	9.8/9.66	101	14	15	11.0/9.66	114
	8	16.7/9.66	173	12	8	10.5/9.66	109
	4	16.2/9.66	167	diethyl malonate	0.6	11.0/9.66	114
	2	14.5/9.66	150	diethyl succinate	0.6	11.6/9.66	120
				malonic acid	0.6	11.0/9.66	114
7	15	10.8/9.66	112	succinic acid	0.6	10.7/9.66	111
	12	10.8/9.66	112	5-fluorouracil	25	18.0/9.66	186
	8	11.9/9.66	124				
8	60	12.7/9.66	131				
	30	15.4/9.66	159				
	15	18.7/9.66	194				
	8	14.0/9.66	145				

[a] Toxic.

(0.3 g) in 15 mL of EtOH was flushed with H_2 and 1.5 mL of 1 M $NaBH_4$ was added by syringe to give a black precipitate. This was left to stir until H_2 uptake ceased. The solution was then cooled to 0 °C in an ice bath. Then, 1 g of 1 in 50 mL of EtOH was added and left until 1 equiv of H_2 was taken up. The catalyst was filtered off and the EtOH was evaporated off in vacuo. The residue was taken up in $CHCl_3$ and extracted with H_2O to remove excess nickel acetate. The $CHCl_3$ layer was dried over $MgSO_4$ and evaporated to give 0.8 g of crude product. This product was recrystallized from acetonitrile four times to give plenolin (6, 500 mg). Its NMR, IR, and melting point were identical to those of an authentic sample of natural plenolin.[13]

2,3-Dihydrohelenalin (9) was prepared according to the procedure of Lee et al.[7]

Catalytic Hydrogenation of 1, 2,3,11,13-Tetrahydrohelenalin (12), and 2,3-Dihydroisohelenalin (15). PtO_2 (150 mg) was added to 393 mg (1.50 mmol) of 1 in 25 mL of EtOAc. After the solution was stirred under hydrogen gas for 5 h, the catalyst was filtered off and the solvent was removed. The residue was column chromatographed over silica gel ($CHCl_3$-EtOH, 10:1) to yield 2,3,11,13-tetrahydrohelenalin (12, 60%), described previously,[7] and compound 15 (30%); colorless needles; mp 206–208 °C; IR ($CHCl_3$) 3400 (OH), 1730 (CO) cm^{-1}; NMR ($CDCl_3$) δ 5.15 (1 H, m, H-8), 4.88 (1 H, br s, H-6), 1.89 (3 H, br s, Me-11), 1.19 (3 H, d, J = 6.0 Hz, Me-10), 0.84 (3 H, s, Me-5); MS m/e 264.1365 (M+) ($C_{15}H_{20}O_4$ requires 264.1361). Compound 15 was identical by TLC and IR, NMR, and mass spectra with that of 2,3-dihydroisohelenalin, obtained from the reduction of isohelenalin.[10]

Bis(helenalinyl) Malonate (2). To 1.0 g (3.82 mmol) of 1 in 10 mL of dry benzene was added 0.8 g (5.67 mmol) of malonyl chloride in 8 mL of dry benzene. The mixture was refluxed for 1 h and then washed with H_2O, aqueous $NaHCO_3$, and saturated NaCl. The organic layer was dried over anhydrous Na_2SO_4 and evaporated to give a crude oil, which was purified by preparative TLC ($CHCl_3$-Me_2CO, 4:1) to give 515 mg of crystalline bis(helenalinyl) malonate (2): mp 217–218 °C; MS, m/e 592.2300 (M^+) ($C_{33}H_{36}O_{10}$ requires 592.2305). The IR and NMR spectral data of 2 have been described previously.[2] Anal. ($C_{33}H_{36}O_{10}$) C, H, O.

Bis(helenalinyl) Succinate (3). To 0.5 g (1.91 mmol) of 1 in 6 mL of dry benzene was added 0.4 g (2.60 mmol) of succinoyl dichloride in 4 mL of dry benzene. The mixture was refluxed for 24 h and worked up in an analogous manner as described above for the preparation of 2 to give a crude oil (0.4 g). This oil was purified by preparative TLC ($CHCl_3$-Me_2CO, 4:1) to afford 150 mg of crystalline bis(helenalinyl) succinate (3): mp 265 °C; IR ($CHCl_3$) 1770 (lactone), 1740 (ester), 1720 (cyclopentenone), 1665, 1590 (C=C) cm^{-1}; NMR ($CDCl_3$) δ 7.69 (2 H, dd, J = 6.0 and 2.0 Hz, H-2 and H-2′), 6.41 (2 H, d, J = 3.0 Hz, H_a and $H_{a'}$), 6.06 (2 H, d, J = 3.0 Hz, H_b and $H_{b'}$), 6.05 (2 H, dd, overlapped m, H-3 and H-3′), 5.37 (2 H, br s, H-6 and H-6′), 4.90 (2 H, m, H-8 and H-8′), 2.50 (4 H, m, $-COCH_2CH_2C-$), 1.30 (6 H, d, J = 6.0 Hz, Me-10 and Me-10′), 1.01 (6 H, s, Me-5 and Me-5′); MS, m/e 606.2471 (M^+) ($C_{34}H_{38}O_{10}$ requires 606.2471). Anal. ($C_{34}H_{38}O_{10}$) C, H, O.

Bis(helenalinyl) Glutarate (4). To 1.0 g (3.82 mmol) of 1 in 10 mL of dry benzene was added 0.8 g (4.73 mmol) of glutaryl dichloride in 8 mL of dry benzene. The mixture was refluxed for 6 h and worked up in a manner similar to that described above for the preparation of 3 to yield a crude oil, which was purified by preparative TLC ($CHCl_3$-Me_2CO, 4:1) to give 300 mg of 4 as a gum: IR (IBr) 1760 (lactone), 1730 (ester), 1710 (cyclopentenone), 1650 (C=C) cm^{-1}; NMR ($CDCl_3$) δ 7.68 (2 H, dd, J = 2.0 and 6.0 Hz, H-2 and H-2′), 6.44 (2 H, d, J = 3.0 Hz, H_a and $H_{a'}$), 6.09 (2 H, d, J = 3.0 Hz, H_b and $H_{b'}$), 6.07 (2 H, overlapped m, H-3 and H-3′), 5.37 (2 H, br s, H-6 and H-6′), 4.89 (2 H, m, H-8 and H-8′), 1.29 (6 H, d, J = 6.0 Hz, Me-10 and Me-10′), 0.99 (6 H, s, Me-5 and Me-5′); MS, m/e 620.2627 (M^+) ($C_{35}H_{40}O_{10}$ requires 620.2622).

Bis(helenalinyl) Adipate (5). Compound 5 was prepared

(13) K. H. Lee, T. Ibuka, A. T. McPhail, K. D. Onan, T. A. Geissman, and T. G. Waddell, *Tetrahedron Lett.*, 1149 (1974).

in an exact manner as described above for the synthesis of 4 by refluxing 1 (1.0 g, 3.82 mmol) with adipoyl dichloride (0.8 g, 4.37 mmol) in dry benzene (18 mL) for 2 h. The crude resulting product was purified by preparative TLC ($CHCl_3$–Me_2CO, 5:1) to give 370 mg of amorphous 5: IR (KBr) 1760 (lactone), 1730 (ester), 1705 (cyclopentenone), 1655 (C=C) cm^{-1}; NMR ($CDCl_3$) δ 7.67 (2 H, dd, J = 2.0 and 6.0 Hz, H-2 and H-2'), 6.44 (2 H, d, J = 3.0 Hz, H_a and $H_{a'}$), 6.12 (2 H, d, J = 3.0 Hz, H_b and $H_{b'}$), 6.06 (2 H, dd, J = 3.0 and 6.0 Hz, H-3 and H-3'), 4.90 (2 H, m, H-8 and H-8'), 1.28 (6 H, d, J = 6.0 Hz, Me-10 and Me-10'), 1.00 (6 H, s, Me-5 and Me-5'); MS, m/e 634.2771 (M^+) ($C_{36}H_{42}H_{10}$ requires 634.2778).

Bis(plenolinyl) Malonate (7). Compound 7 was prepared according to the aforementioned procedure described for the synthesis of 2 by refluxing plenolin (6; 0.5 g, 1.89 mmol) and malonyl dichloride (0.45 g, 3.21 mmol) in dry benzene (15 mL) for 1.5 h. The resulting pale yellow oil was purified by preparative TLC to yield 7 as colorless crystals (480 mg): mp 247–249 °C dec; IR (KBr) 1765 (lactone), 1735 (ester), 1700 (cyclopentenone) cm^{-1}; NMR ($CDCl_3$) δ 7.68 (2 H, dd, J = 6.0 and 2.0 Hz, H-2 and H-2'), 6.0 (2 H, dd, J = 6.0 and 3.0 Hz, H-3 and H-3'), 5.45 (2 H, s, H-6 and H-6'), 4.77 (2 H, m, H-8 and H-8'), 3.12 (2 H, s, $-COCH_2CO-$), 1.48 (6 H, d, J = 6.0 Hz, Me-11 and Me-11'), 1.25 (6 H, d, J = 6.0 Hz, Me-10 and Me-10'), 1.04 (6 H, s, Me-5 and Me-5'); MS, m/e 596.2627 (M^+) ($C_{33}H_{40}O_{10}$ requires 596.2622).

Bis(plenolinyl) Succinate (8). Treatment of 6 (0.5 g, 1.89 mmol) with succinoyl dichloride (0.4 g, 2.58 mmol) in dry benzene (10 mL) by refluxing the mixture for 72 h in a similar manner as described above for the preparation of 3 afforded a crude oil. Purification of this oil by preparative TLC ($CHCl_3$–Me_2CO, 5:1) gave 275 mg of amorphous 8: IR ($CHCl_3$) 1780 (lactone), 1740 (ester), 1723 (cyclopentenone) cm^{-1}; NMR ($CDCl_3$) δ 7.74 (2 H, dd, J = 6.0 and 2.0 Hz, H-2 and H-2'), 6.06 (2 H, dd, J = 6.0 and 3.0 Hz, H-3 and H-3'), 5.44 (2 H, s, H-6 and H-6'), 4.77 (2 H, m, H-8 and H-8'), 2.43 (4 H, m, $-COCH_2CH_2CO-$), 1.47 (6 H, d, J = 6.0 Hz, Me-11 and Me-11'), 1.23 (6 H, d, J = 6.0 Hz, Me-10 and Me-10'), 1.02 (6 H, s, Me-5 and Me-5'); MS, m/e 610.2772 (M^+) ($C_{34}H_{42}O_{10}$ requires 610.2778).

Treatment of 2,3-Dihydrohelenalin (9) with Malonyl Dichloride. Bis(2,3-dihydrohelenalinyl) Malonate (10) and 2,3-Dihydrohelenalinyl Ethyl Malonate (14). To a solution of 9 (532 mg, 2.02 mmol) in dry benzene (15 mL) was added a solution of malonyl dichloride (400 mg, 2.86 mmol) in dry benzene (10 mL). This mixture was refluxed for 1 h, then washed with $NaHCO_3$ and H_2O, dried ($MgSO_4$), and evaporated in vacuo to give a pale yellow oil (500 mg), which was subjected to preparative TLC ($CHCl_3$–EtOH, 20:1) to yield compounds 10 and 14. Compound 10 (140 mg, colorless crystals): mp 114–116 °C; IR (KBr) 1755 (lactone), 1740 (ester) cm^{-1}; NMR ($CDCl_3$) δ 6.40 (2 H, d, J = 3.0 Hz, H_a and $H_{a'}$), 6.03 (2 H, d, J = 3.0 Hz, H_b and $H_{b'}$), 5.38 (2 H, d, J = 2.0 Hz, H-6 and H-6'), 4.81 (2 H, m, H-8 and H-8'), 3.56 (2 H, m, H-7 and H-7'), 3.29 (2 H, s, $-COCH_2CO-$), 1.06 (6 H, d, J = 6.0 Hz, Me-10 and Me-10'), 0.77 (6 H, s, Me-5 and Me-5'); MS, m/e 596.2627 (M^+) ($C_{33}H_{40}O_{10}$ requires 596.2622). Compound 14: IR (neat) 1775–1720 (lactone, ester and cyclopentanone) cm^{-1}; NMR ($CDCl_3$) δ 6.40 (1 H, d, J = 3.0 Hz, H_a), 6.05 (1 H, d, J = 3.0 Hz, H_b), 5.32 (1 H, d, J = 2.0 Hz, H-6), 4.80 (1 H, m, H-8), 4.19 (2 H, q, J = 7.0 Hz, $COCH_2CH_3$), 3.59 (1 H, m, H-7), 3.35 (2 H, s, $-COCH_2CO-$), 1.29 (3 H, t, J = 7.0 Hz, $-COOCH_2CH_3$), 1.05 (3 H, d, J = 6.0 Hz, Me-10), 0.78 (3 H, s, Me-5); MS, m/e 378.1673 (M^+) ($C_{20}H_{26}O_7$ requires 378.1678).

Bis(2,3-dihydrohelenalinyl) Succinate (11). Compound 11 was prepared in an analogous manner as described above for the synthesis of 8. The crude oil resulting from a 36-h reflux of 9 (0.5 g, 1.89 mmol) with succinoyl dichloride (0.4 g, 2.58 mmol) in dry benzene (10 mL) was purified by preparative TLC ($CHCl_3$–Me_2CO, 4:1) to give 250 mg of amorphous 11: IR ($CHCl_3$) 1765 (lactone), 1752 (ester and cyclopentanone), 1669 (C=C) cm^{-1}; NMR ($CDCl_3$) δ 6.47 (2 H, d, J = 3.0 Hz, H_a and $H_{a'}$), 6.08 (2 H, d, J = 3.0 Hz, H_b and $H_{b'}$), 5.31 (2 H, d, J = 2 Hz, H-6 and H-6'), 4.84 (2 H, m, H-8 and H-8'), 3.56 (2 H, m, H-7 and H-7'), 2.55 (4 H, m, $-COCH_2CH_2CO-$), 1.09 (6 H, d, J = 6.0 Hz, Me-10 and Me-10'), 0.78 (6 H, s, Me-5 and Me-5'); MS, m/e 610.2772 (M^+) ($C_{34}H_{42}O_{10}$ requires 610.2778).

Bis(2,3,11,13-tetrahydrohelenalinyl) Malonate (13). Compound 13 (285 mg) was obtained from an analogous method described above by reacting 12 (266 mg, 1 mmol) and malonyl dichloride (200 mg, 1.43 mmol): mp 118–120 °C; IR (KBr) 1750 (lactone), 1735 (ester) cm^{-1}; NMR ($CDCl_3$) δ 5.39 (2 H, s, H-6 and H-6'), 4.72 (2 H, m, H-8 and H-8'), 3.18 (2 H, s, $-COCH_2CO-$), 1.44 (6 H, d, J = 6.0 Hz, Me-11 and Me-11'), 1.07 (6 H, d, J = 6.0 Hz, Me-10 and Me-10'), 0.86 (6 H, s, Me-5 and Me-5'); MS, m/e 600.2931 (M^+) ($C_{33}H_{44}O_{10}$ requires 600.2931).

Biological Methods. The antileukemic activity test against the lymphocytic leukemia P-388 maintained in our laboratory at UNC[2] was conducted in BDF_1 male mice (~22 g). In this screen, 10^6 cells were implanted on day 0. The test compounds were administered intraperitoneally from 0.6 to 60 (mg/kg)/day for 2 weeks. T/C values were calculated according to the NIH protocol.[11] 5-Fluorouracil was used as the internal standard in the screen.

Acknowledgment. This investigation was generously supported by Grants CA 17625 from the National Cancer Institute, NIH (K.H.L.), and the Texas Agricultural Experiment Station, Texas A & M University System Project No. H-6255 (H.L.K.). We thank Dr. David Rosenthal and Fred Williams of the Research Triangle Center for Mass Spectrometry for mass spectral data.

Gastric Antisecretory Agents. 1. Antisecretory and Antiulcer Activity of 5*H*-[1]Benzopyrano[2,3-*b*]pyridin-5-ylureas and 5*H*-[1]Benzothiopyrano[2,3-*b*]pyridin-5-ylureas

James A. Bristol,*[1] Elijah H. Gold, Raymond G. Lovey,

Department of Chemical Research

and James F. Long

Department of Pharmacology, Pharmaceutical Research Division, Schering-Plough Corporation, Bloomfield, New Jersey 07003. *Received September 8, 1980*

5*H*-[1]Benzopyrano[2,3-*b*]pyridin-5-ylureas and 5*H*-[1]benzothiopyrano[2,3-*b*]pyridin-5-ylureas are a new class of gastric antisecretory agents and antiulcer agents. Certain compounds inhibit histamine-, dimaprit-, insulin-, and food-stimulated gastric acid secretion in dogs, as well as aspirin-induced ulcers in rats. Most compounds are antisecretory in the pylorus-ligated rat. Several compounds are comparably potent to cimetidine.

The introduction and wide acceptance of the histamine (H_2) receptor antagonist cimetidine[2] for the treatment of peptic ulcer disease has renewed interest in the drug therapy of this disease. The effectiveness of the histamine

0022-2623/81/1824-0927$01.25/0