Antitumor Agents. 11.[1] Synthesis and Cytotoxic Activity of Epoxides of Helenalin Related Derivatives

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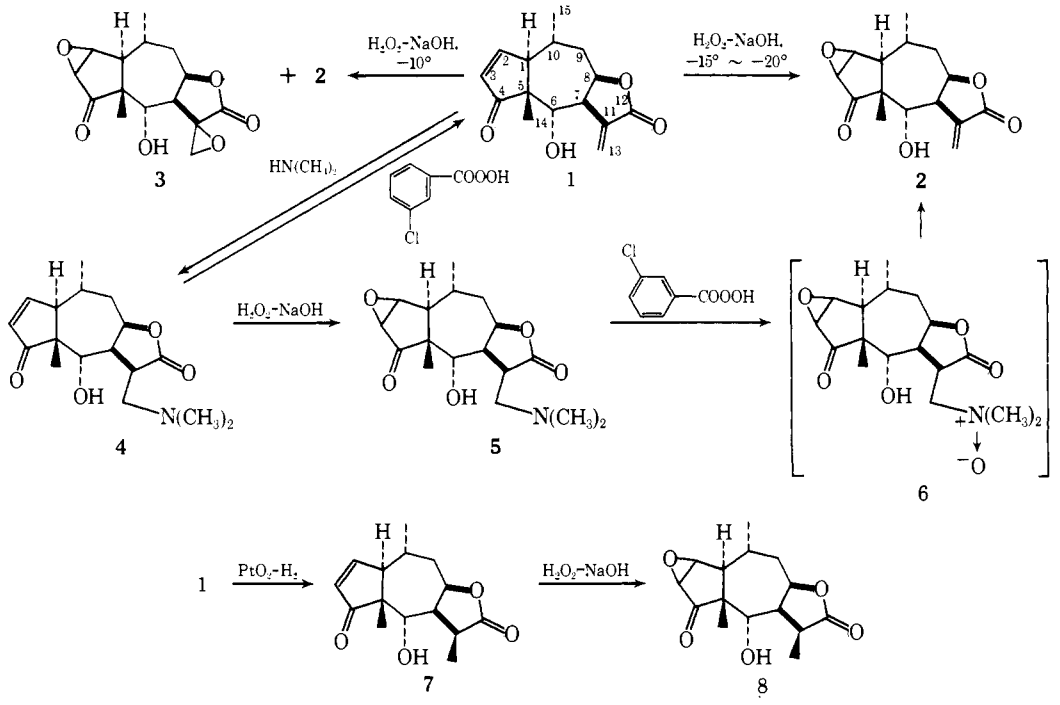
Several epoxides of helenalin related derivatives have been synthesized in an effort to evaluate the potential significance of the epoxycyclopentanone moiety for cytotoxic activity against the growth of tissue culture cells originating from human epidermoid carcinoma of larynx (H.Ep.-2). Helenalin (1) was converted to the monoepoxy derivative 2 and the diepoxy derivative 3 by alkaline hydrogen peroxide at different temperatures. Alternative synthesis of 2 was achieved by a convenient method of protecting the α-methylene grouping of the γ-lactone, *i.e.*, epoxidation of helenalin dimethylamine adduct 4, followed by treatment of the reaction product 5 with *m*-chloroperbenzoic acid. 2,3-Epoxy-11,13-dihydrohelenalin (8) was prepared by direct epoxidation of 11,13-dihydrohelenalin (7). Treatment of mexicanin A (9) with *m*-chloroperbenzoic acid gave, in addition to the 1,2-epoxy derivative 10, 1-α-hydroxyhelenalin (11) which furnished an acetate (12) upon acetylation. Catalytic hydrogenation of 10 yielded the dihydroepoxide 13. Treatment of 1 or acetylhelenalin (15) with Ac_2O–*p*-TsOH gave the same acetyl dienol acetate (14). Epoxidation of 14 with *m*-chloroperbenzoic acid gave 1,β-hydroxyhelenalin (19) and a mixture of monoepoxides (17 and 18) which yielded 19 and 11 upon silica gel chromatography. The results of the cytotoxicity test of the compounds studied indicate that either an α- or a β-epoxycyclopentanone moiety in helenalin related derivatives contributes significantly to the cytotoxicity. Furthermore, this cytotoxicity appears to be independent of the presence or absence of an α-epoxy-γ-lactonic moiety.

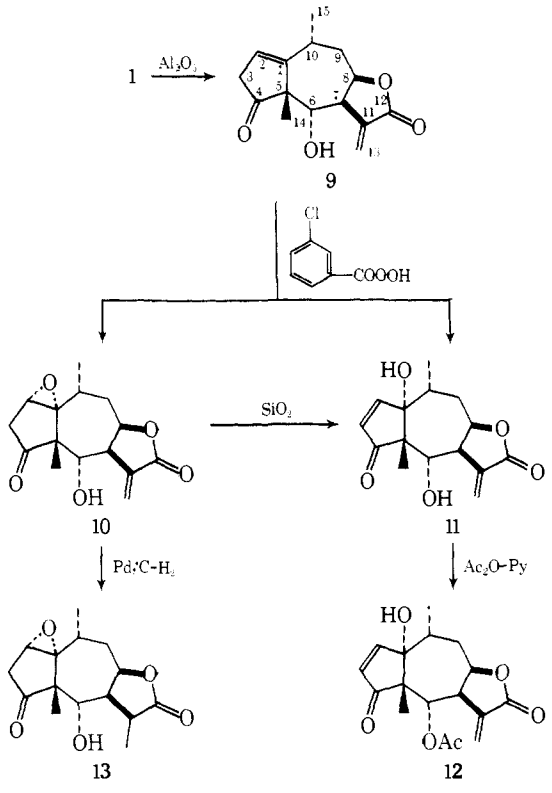
In a preliminary communication,[2] the effect of epoxidation on the cytotoxicity of helenalin (1) related derivatives has been reported. It was found that an α-epoxycyclopentanone moiety of helenalin derivatives, such as in 2,3-epoxyhelenalin (2), contributes in a significant manner to the cytotoxicity. In this paper, we have extended our investigation of synthetic compounds containing an epoxy function β to the cyclopentanone carbonyl of helenalin related derivatives in order to determine whether such modification affects the potential cytotoxic activity. The preparation of epoxides α to the cyclopentanone carbonyl of helenalin related derivatives is also presented.

Chemistry. The syntheses of epoxides α to the cyclopentanone carbonyl of helenalin related derivatives are shown in Scheme I. 2,3-Epoxyhelenalin (2) was prepared in 79% yield by selective epoxidation of helenalin (1) with a hydrogen peroxide–sodium hydroxide solution at −15°. Epoxidation of 1 according to the procedure of Adams and Herz[3] resulted in the formation of a 2:1 mixture of 2 and 2,3,11,13-diepoxyhelenalin (3), from which 3 was isolated. The 5–10° difference in the reaction temperature for achieving selective epoxidation of 1 is noteworthy since the procedures for making 2 and for 2 and 3 are quite similar. An alternative method for the synthesis of 2 involved epoxidation of the Michael-type dimethylamine adduct 4 of helenalin with alkaline hydrogen peroxide yielding the corresponding epoxide 5. The subsequent conversion of 5 to 2 was obtained in quantitative yield by treatment with *m*-chloroperbenzoic acid probably *via* a possible *N*-oxide intermediate 6, thereby providing a convenient method

Scheme I



Scheme II



for the protection of the highly reactive α-methylene grouping of the γ-lactone. As described previously,[4] the generation of the latter moiety usually required the quaterization of the dimethylamino group with methyl iodide followed by elimination of the alkylated (tertiary) amine. Similar conversion of **4** to **1** by treatment with *m*-chloroperbenzoic acid was effected in quantitative yields. Compound **8** was also prepared from **7** by epoxidation with alkaline hydrogen peroxide in order to clarify the role of the α-epoxy-γ-lactonic moiety, such as in **3**, with respect to the effect upon cytotoxicity. Compound **7** (11,13-dihydrohelenalin[4,5] or plenolin[6]) was prepared according to a procedure described in the literature.[5]

The β-epoxycyclopentanones of helenalin related derivatives, such as 1,2-epoxymexicanin A (**10**) and 11,13-dihydro-1,2-epoxymexicanin A (**13**), were synthesized from mexicanin A (**9**)[7] which was obtained by treatment of **1** with deactivated neutral alumina in chloroform[8] as shown in Scheme II. Treatment of **9** with *m*-chloroperbenzoic acid gave, in addition to the expected 1,2-epoxy derivative **10**, a rearranged product 1-α-hydroxyhelenalin (**11**). The following evidence is supportive of structure **11**: the composition of $C_{15}H_{18}O_5$, the prominence of a strong absorption band corresponding to the cyclopentenone carbonyl group at 1713 cm^{-1} in the ir spectrum, and the appearance of a pair of AB-type doublets at δ 7.70 (1 H, H-2, J = 6.0 Hz) and 6.10 (1 H, H-3, J = 6.0 Hz) in the nmr spectrum. Compound **11** could also be obtained by chromatography of **10** with silica gel in $CHCl_3$–EtOAc (1:1). Acetylation of **11** with acetic anhydride in pyridine led to the formation of 1α-hydroxyhelenalin acetate (**12**) in quantitative yield. Catalytic hydrogenation of **10** with palladium on carbon afforded the corresponding dihydro derivative **13**.

Encouraged by the significant cytotoxicities exhibited by all of the epoxycyclopentanone derivatives so far prepared, we sought next to prepare a diepoxide derivative, such as **16**, since it is well known that many naturally occurring substances owe their antitumor–cytotoxic activity to introduction of the diepoxide functionality as described previously.[2] Upon treatment with *p*-toluenesulfonic acid in acetic anhydride, helenalin (**1**) was converted to an acetyl dienol acetate **14** in quantitative yield. The pres-

Scheme III

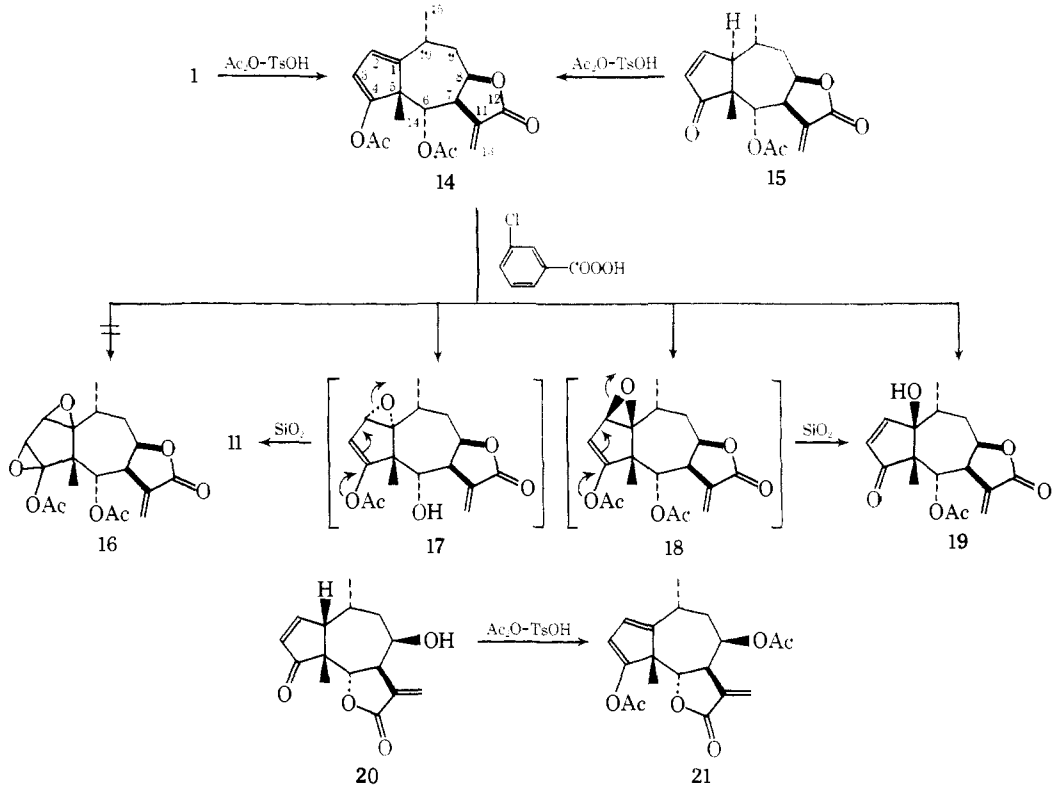


Table I. Cytotoxicity of Epoxides and Related Helenalin Derivatives

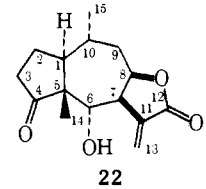
No.	Formula	Analyses[a]	Mp, °C	Recrystn solvent	Ir, cm^{-1} [b]	ED_{50}, $\mu g/ml$[c] (H.Ep.-2)
1	$C_{15}H_{18}O_4$		170–172[d]	Benzene		0.10
2	$C_{15}H_{18}O_5$	C, H	217 dec	MeOH	3425, 1755, 1740, 1660	0.11
3	$C_{15}H_{18}O_6$	C, H	234–236	MeOH	3460, 1765, 1738	0.50
4	$C_{17}H_{25}O_4N$		214 dec[e]	$CHCl_3$–EtOH		0.60
5	$C_{17}H_{25}O_5N$	C, H	194–195	Et_2O–EtOAc	3460, 1770, 1740	0.92[f]
7	$C_{15}H_{20}O_4$	C, H	223–226[g]	CH_3CN	3450, 1745, 1712	0.81
8	$C_{15}H_{20}O_5$	C, H	218	$CHCl_3$–Et_2O	3560, 1770, 1740[h]	0.39
9	$C_{15}H_{18}O_4$		140–142[i]	Me_2CO–Et_2O		2.30
10	$C_{15}H_{18}O_5$	C, H	173 dec	$CHCl_3$–Et_2O	3470, 1768, 1755, 1660[j]	0.53
11	$C_{15}H_{18}O_5$	C, H	148–150	$CHCl_3$–Et_2O	3300, 1740, 1713, 1665	0.27
12	$C_{17}H_{20}O_6$	C, H	168–170	CH_2Cl_2–Et_2O	3500, 1760, 1720, 1710, 1660	
13	$C_{15}H_{20}O_5$	C, H	192 dec	$CHCl_3$–Et_2O	3480, 1760[h,k]	0.26
14	$C_{19}H_{22}O_6$	C, H	145–148	MeOH	1765, 1730, 1660, 1618	0.78
15	$C_{17}H_{20}O_5$		180–180.5[l]	CH_2Cl_2–Et_2O		0.29
19	$C_{17}H_{20}O_6$	C, H	165–166	$CHCl_3$–Et_2O	3480, 1770, 1740, 1710, 1660[h]	0.65
20	$C_{15}H_{18}O_4$		179–180[m]	CH_2Cl_2–Et_2O		
21	$C_{19}H_{22}O_6$	C, H	188–190	CH_2Cl_2–Et_2O	1765, 1730, 1660, 1625	
22	$C_{15}H_{20}O_4$		154–155[e]	CH_2Cl_2–hexane		3.84
23	$C_{15}H_{22}O_4$		171–173[e]	Ethyl *n*-butyrate		>40.00

[a] Where analyses are indicated only by symbols of the elements, analytical results obtained for these elements were within ±0.4% of the theoretical values. [b] Taken in Nujol. [c] The values of ED_{50} are used for expressing the potency of cytotoxicity which is the calculated effective dose that inhibits the net cell growth to 50% of control growth. [d] See ref 9. [e] See ref 4. [f] The ED_{50} value of 5 (1.36) reported in ref 2 is incorrect and has to be changed to 0.92. [g] See ref 4–6. [h] Taken in chloroform. [i] See ref 7. [j] Taken in KBr. [k] Double strength intensity. [l] See ref 10. [m] See ref 8.

ence of an acetyl dienol acetate system in **14** was indicated by the appearance of a strong uv absorption at 268 nm and was substantiated by the presence in the nmr spectrum of a pair of low-field doublets at δ 6.10 (1 H, H-3, J = 3.0 Hz) and 6.00 (1 H, H-2, J = 3.0 Hz) as well as two singlets at 2.21 (3 H, C_4-OAc) and 2.01 (3 H, C_6-OAc). Further evidence for the establishment of structure **14** for this acetyl dienol acetate was provided by the fact that **14** could also be prepared from helenalin acetate (**15**).[9,10] Furthermore, a similar treatment of 1-epiallohelenalin (**20**) with *p*-toluenesulfonic acid in acetic anhydride led to the formation of the corresponding acetyl dienol acetate **21**. Epoxidation of **14** with *m*-chloroperbenzoic acid gave, instead of the expected diepoxide **16**, a mixture of monoepoxides and a hydroxylcyclopentenone derivative **19**. Structures **17** and **18** were assigned for the mixture since upon chromatography on silica gel it generated **11** and **19**, respectively. Compound **19** had the composition of $C_{17}H_{20}O_6$ and showed the presence of a hydroxyl group (3480 cm^{-1}) and a γ-lactone ring (1770 cm^{-1}). The presence of a cyclopentenone ring system in **19** was indicated by the appearance of an ir band at 1710 cm^{-1} and was confirmed by the presence in the nmr spectrum of the characteristic pair of low-field doublets at δ 7.35 (1 H, J = 6.0 Hz) and 6.40 (1 H, J = 6.0 Hz) (Scheme III).

The differences between structures **12** and **19** are apparently due to the dissimilar configuration of the C-1 hydroxyl groups in both compounds. A comparison of the chemical shifts for the α-C_8 proton in the nmr spectra of **11** and **19** lead to the conclusion that the C_1-OH group in **12** is α and in **19** is β oriented. The α-H_8 in **19** appears at δ 4.37 (1 H, m) whereas in **12** it is found at the lower field at δ 5.40 (1 H, m) which is deshielded by the presence of an adjacent C_1-α-OH as shown by Drieding models. The presence of an ir band at 3300 cm^{-1} which is due to the hydrogen bonding between the adjacent C_6-α-OH and C_1-α-OH in **11** is also in keeping with this assignment. Considerations from the above evidence as well as the reaction mechanism (see structures **17** and **18**) led to the conclusion that the configuration of the epoxides in **10**, **13**, and **17** could be assigned as α and that in **18** as β.

Cytotoxicity and Structure–Activity Relationships. The epoxy derivatives and related compounds prepared in this study were assayed for their cytotoxicity against the growth of tissue culture cells originating from human epidermoid carcinoma of larynx (H.Ep.-2) according to a rapid microtiter method previously described.[11] A comparison of the ED_{50} values for the cytotoxicity of the compounds listed in Table I disclosed that both the 2,3 double bond of compound **1** and the 2,3-epoxide of compound **2** gave equally effective cytotoxicity. The 1,2-epoxide of compound **10** is five times less active in comparison with **1** and **2**. The corresponding saturated compound **22** gave a 35-fold decrease in activity. Significant cytotoxicity could



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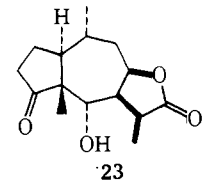

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also be maintained when the two potential alkylating centers, such as the O=C—C=CH_2 system in the ketone and the lactone of helenalin (**1**), were masked by the epoxy moiety, although the diepoxide **3** was five times less active in comparison with **1**. However, the absence of the diepoxy functionality in **3** resulted in more than an 80-fold diminution in cytotoxicity (compare **3** and **23**). The fact that practically identical levels of cytotoxicity of **3** were obtained as compared to **5** and **8** would indicate that the α-epoxy-γ-lactonic moiety made no contribution to cytotoxic activity. Moreover, a comparison of the activities of compounds **8** and **13** to compounds **4**, **7**, and **23** further confirmed that an epoxy function either α or β to the cy-

clopentanone carbonyl in helenalin related derivatives contributes significantly to the cytotoxicity. It has been demonstrated by the *in vivo* Ehrlich ascites screen that compounds **2** and **10** are active.[12]

Experimental Section

Unless otherwise specified, melting points were determined on a Thomas-Hoover melting point apparatus and are corrected. Ir spectra were determined in Nujol mulls with a Perkin-Elmer 257 grating ir spectrophotometer. Uv spectra were determined in 95% EtOH with a Cary Model 15 spectrophotometer. Nmr spectra were measured in $CDCl_3$ with a Jeolco C-60 HL spectrometer (TMS), and chemical shifts reported in δ (ppm) units: s, singlet; d, doublet; t, triplet; m, multiplet; and dt, doublets of triplets; and the J values in hertz. Mass spectra were determined on an A.E.I. MS-902 instrument at 70 eV using a direct inlet system. Silica gel for column chromatography refers to Baker A.R. No. 3405; silica gel for the preparative tlc refers to Merck silica gel GF-254; and silica gel for the tlc refers to Merck silica gel G developed with chloroform–acetone (3:1) and visualized by spraying with 40% aqueous sulfuric acid and heating. Deactivated neutral alumina refers to neutral alumina AG-7 (100–200 mesh), Bio-Rad, activity grade III. Elemental analyses were performed by Atlantic Microlab, Inc., Atlanta, Ga.

2,3-Epoxyhelenalin (2). A solution of helenalin (1) (1 g, 3.78 mmol) in MeOH (30 ml) was cooled to −15 to −20° and 9 ml of a mixture of 30% H_2O_2 (1 ml), 4 *N* NaOH (1 ml), H_2O (1 ml), and MeOH (15 ml) was added dropwise to the solution. After 10 min the solution was diluted with ice water (50 ml) and extracted with $CHCl_3$. The $CHCl_3$ extract was dried (Na_2SO_4) and evaporated *in vacuo* to afford a colorless crystalline residue (840 mg, 79%) which was recrystallized from MeOH to yield **2**: mp 217° dec; nmr (DMSO-d_6) 6.18 (1 H, d, J = 3.0, H-13), 5.80 (1 H, d, J = 3.0, H-13), 5.02 (1 H, dt, J = 3.0, 7.3, H-8), 1.32 (3 H, d, J = 6.0, H-15), and 0.98 (3 H, s, H-14).

2,3,11,13-Diepoxyhelenalin (3). Epoxidation of 1 (500 mg, 1.89 mmol) in MeOH (15 ml) with a solution of 30% H_2O_2 (0.5 ml), H_2O (0.5 ml), and 4 *N* NaOH (0.5 ml) at −10° according to the procedure described previously[3] gave colorless prisms: mp 204–206° (ref 3 reported mp 215–216° after repeated recrystallization from MeOH). The nmr spectrum of this compound indicated that it is a 2:1 mixture of the monoepoxide **2** and the diepoxide **3**. Attempted isolation of **2** from the mixture was unsuccessful. The mother liquor after the removal of the crystalline mixture was diluted with H_2O and extracted with $CHCl_3$. The $CHCl_3$ extract was dried (Na_2SO_4) and evaporated to a crystalline residue. Recrystallization from MeOH gave **3** (95 mg, 17%) as colorless prisms: mp 234–236°; nmr (DMSO-d_6) 5.17 (1 H, m, H-8), 1.28 (3 H, d, J = 6.0, H-15), and 1.02 (3 H, s, H-14).

2,3-Epoxyhelenalin Dimethylamine Adduct 5. A solution of helenalin dimethylamine adduct **4** (50 mg, 0.16 mmol), prepared according to a procedure described in ref 4, in MeOH (10 ml) was cooled to −10° and a mixture of 30% H_2O_2 (0.5 ml), H_2O (0.5 ml), and 4 *N* NaOH (0.5 ml) was added dropwise to the solution. After 10 min the mixture was diluted with H_2O (30 ml) and extracted with $CHCl_3$ (60 ml). The $CHCl_3$ extract was dried (Na_2SO_4) and evaporated to yield, after recrystallization from Et_2O–EtOAc, 35 mg (67%) of **5** as colorless prisms: mp 194–195°; nmr 4.90 (1 H, m, H-8), 2.27 [6 H, s, $N(CH_3)_2$], 1.32 (3 H, d, J = 6.0, H-15), and 1.22 (3 H, s, H-14).

Treatment of 2,3-Epoxyhelenalin Dimethylamine Adduct 5 with *m*-Chloroperbenzoic Acid. Compound 2. A solution of compound **5** (100 mg, 0.31 mmol) in $CHCl_3$ (5 ml) was treated with a solution of *m*-chloroperbenzoic acid (100 mg) in $CHCl_3$ (5 ml) and the mixture allowed to stand at 5° overnight. The solution was washed with cold 5% aqueous $NaHCO_3$ and with H_2O, dried, and evaporated *in vacuo* to give, after recrystallization from MeOH, a quantitative yield of compound **2**. The identity of **2** was established by direct comparison with a sample of 2,3-epoxyhelenalin, obtained by selective epoxidation of 1 described previously, by mixture melting point, tlc, and superimposable ir spectra.

Treatment of Helenalin Dimethylamine Adduct 4 with *m*-Chloroperbenzoic Acid. Compound 1. Treatment of **4** (100 mg, 0.33 mmol) with *m*-chloroperbenzoic acid (150 mg) in $CHCl_3$ (10 ml) in a similar manner as described above for the conversion of **5** to **2** afforded 1 in quantitative yield. The identity of 1 with helenalin was established by tlc, ir comparison, and mixture melting point determination.

11,13-Dihydrohelenalin (7). Compound 7 was prepared by catalytic hydrogenation of 1 with PtO_2–EtOAc according to the method of Adams and Herz[5] and purified by repeated recrystallization with CH_3CN until its nmr spectrum showed completely identical with that of plenolin.[6]

2,3-Epoxy-11,13-dihydrohelenalin (8). A solution of 7 (105 mg, 0.39 mmol) in MeOH (15 ml) was cooled to 0° and treated with 1 ml of a mixture of 30% H_2O_2 (1 ml), 4 *N* NaOH (1 ml), H_2O (1 ml), and MeOH (15 ml). After 1 hr the solution was diluted with 30 ml of ice water and extracted with $CHCl_3$. The $CHCl_3$ layer was dried and evaporated to yield a colorless crystalline residue (95 mg, 85%). Recrystallization from $CHCl_3$–Et_2O gave **8**: mp 218° (slowly sintered at 183°); nmr 4.73 (1 H, m, H-8), 1.38–1.25 (6 H, overlapped doublets, H-13 and H-15), and 1.05 (3 H, s, H-14).

Treatment of Mexicanin A (9) with *m*-Chloroperbenzoic Acid. 1,2-Epoxymexicanin A (10) and 1α-Hydroxyhelenalin (11). Treatment of **9** (450 mg, 1.71 mmol), prepared from reaction of 1 with deactivated neutral alumina according to ref 8, with *m*-chloroperbenzoic acid (450 mg) in $CHCl_3$ (10 ml) in an analogous manner as described for the conversion of **5** to **2** gave a colorless crystalline residue which was recrystallized from $CHCl_3$–Et_2O to give **10** (250 mg, 52%): mp 173° dec; nmr 6.39 (1 H, d, J = 3.0, H-13), 5.90 (1 H, d, J = 3.0, H-13), 5.00 (1 H, m, H-8), 4.36 (1 H, d, J = 4.5, H-6), 0.99 (3 H, s, H-14), and 0.95 (3 H, d, J = 6.0, H-15).

The mother liquor after the isolation of **10** was purified by preparative tlc [silica gel, $CHCl_3$–Me_2CO (3:1)] to afford **11** as colorless crystals (60 mg, 13%) upon recrystallization from $CHCl_3$–Et_2O: mp 148–150°; nmr 7.70 (1 H, d, J = 6.0, H-2), 6.10 (1 H, d, J = 6.0, H-3), 6.35 (1 H, d, J = 3.0, H-13), 5.73 (1 H, d, J = 3.0, H-13), 5.45 (1 H, m, H-8), 1.27 (3 H, d, J = 6.0, H-15), and 1.13 (3 H, s, H-14).

Compound **11** was also obtained in 40% yield when 100 mg of **9** was treated with *m*-chloroperbenzoic acid (85 mg, 18%) in CH_2Cl_2 (5 ml) at room temperature overnight, followed by silica gel column chromatography [$CHCl_3$–EtOAc (1:1)] of the reaction products.

1α-Hydroxyhelenalin Acetate (12). Treatment of **11** (20 mg, 0.07 mmol) with Ac_2O–pyridine in the usual way afforded the acetate **12**: colorless crystals from CH_2Cl_2–Et_2O in quantitative yield; mp 168–170°; nmr 7.74 (1 H, d, J = 6.0, H-2), 6.13 (1 H, d, J = 6.0, H-3), 6.49 (1 H, d, J = 3.0, H-13), 6.14 (1 H, d, J = 3.0, H-13), 5.73 (1 H, s, H-6), 5.40 (1 H, m, H-8), 3.45 (1 H, m, H-7), 2.03 (3 H, s, $OCOCH_3$), 1.32 (3 H, overlapped d, H-15), and 1.24 (3 H, s, H-14).

1,2-Epoxy-11,13-dihydromexicanin A (13). Compound **10** (30 mg, 0.11 mmol) in MeOH (10 ml) was hydrogenated in the presence of prereduced 5% Pd/C (10 mg) at room temperature and atmospheric pressure. After removal of the catalyst, the crystalline residue was recrystallized from $CHCl_3$–Et_2O to give **13** in quantitative yield: mp 192° dec (slowly sintered at 163°); nmr 4.92 (1 H, m, H-8), 1.34 (3 H, d, J = 6.0), 0.91 (3 H, d, J = 6.5) (two secondary CH_3), and 1.09 (3 H, s, H-14); *m/e* 280.1312 (M^+).

Treatment of Helenalin (1) with Acetic Anhydride-*p*-Toluenesulfonic Acid. Compound 14. A mixture of 1 (500 mg, 1.89 mmol) and *p*-TsOH (100 mg) in Ac_2O (40 ml) was distilled over 5 hr until most of acetic acid and acetic anhydride were evaporated (bath temperature 150°). A dark-brown oily residue was then taken up in Et_2O (30 ml). The Et_2O layer was washed with H_2O, dried (anhydrous Na_2SO_4), and evaporated under vacuum to yield a yellowish oil. The oil was crystallized from MeOH to afford **14** as colorless crystals in quantitative yield: mp 145–148°; nmr 6.29 (1 H, d, J = 3.0, H-13), 5.78 (1 H, d, J = 3.0, H-13), 6.10 (1 H, d, J = 3.0, H-3), 6.00 (1 H, d, J = 3.0, H-2), 5.35 (1 H, d, J = 9.0, H-6), 4.55 (1 H, m, H-8), 2.21 (3 H, s, C_4-$OCOCH_3$), 2.01 (3 H, s, C_6-$OCOCH_3$), 1.32 (1 H, d, J = 6.0, H-15), and 1.20 (3 H, s, H-14).

Compound **14** was also obtained quantitatively from an analogous method described above by reaction of acetylhelenalin (**15**, 700 mg, 2.30 mmol) and a mixture of *p*-TsOH (50 mg) and Ac_2O (30 ml).

Treatment of 1-Epiallohelenalin (20) with Acetic Anhydride-*p*-Toluenesulfonic Acid. Compound 21. Compound **20** (50 mg, 0.19 mmol) in Ac_2O (6 ml) was refluxed with *p*-TsOH (5 mg). After 5 hr, the reaction mixture was distilled off *in vacuo*. The residue was added with Ac_2O (6 ml) and further refluxed for 5 hr. The reaction product was worked up as described previously to give **21** as colorless crystals in quantitative yield after one recrystallization from CH_2Cl_2–Et_2O: mp 188–190°; nmr 6.23 (1 H, d, J = 3.0, H-13), 5.48 (1 H, d, J = 3.0, H-13), 6.19 (1 H, overlapped d, J = 3.0, H-3), 6.07 (1 H, d, J = 3.0, H-2), 4.72 (1 H, d, J = 9.0,

H-6), 2.20 (3 H, s, C_4-$OCOCH_3$), 2.02 (3 H, s, C_8-$OCOCH_3$), 1.52 (3 H, s, H-14), and 1.22 (3 H, d, J = 6.0, H-15).

Treatment of 14 with *m*-Chloroperbenzoic Acid. 1β-Hydroxyhelenalin Acetate (19) and 1α-Hydroxyhelenalin (11). A solution of 14 (586 mg, 1.69 mmol) and *m*-chloroperbenzoic acid (400 mg) in $CHCl_3$ (50 ml) was allowed to stand at room temperature overnight. The reaction mixture was washed with 5% Na_2SO_3, 5% $NaHCO_3$, and H_2O, dried (Na_2SO_4), and evaporated *in vacuo* to give a yellowish oil. This was chromatographed on Florisil (Floridin Co., 100–200 A mesh, 1.3 × 30 cm) with benzene and $CHCl_3$ as the eluting solvents. The benzene fractions (50 ml) yielded, after evaporation, a pale yellowish oil (360 mg) which showed only a faster moving single spot on tlc. The nmr spectrum, however, indicated that this was a 1:1 mixture of two components. The subsequent $CHCl_3$ fractions (50 ml) gave, after evaporation, a colorless crystalline residue (160 mg, 29%) which corresponded to the slower moving spot on tlc. Recrystallization from $CHCl_3$–Et_2O furnished **19**: mp 165–166°; nmr 7.35 (1 H, d, J = 6.0, H-2), 6.40 (1 H, d, J = 6.0, H-3), 6.22 (1 H, br s, H-13), 5.72 (1 H, br s, H-13), 5.25 (1 H, d, J = 10.5, H-6), 4.37 (1 H, m, H-8), 3.70 (1 H, m, H-7), 2.10 (3 H, s, C_6-$OCOCH_3$), 1.21 (3 H, d, J = 6.8, H-15), and 1.18 (3 H, s, H-14).

Attempted separation of the foregoing 1:1 mixture by silica gel column chromatography in $CHCl_3$ led to the isolation of two crystalline solids which were identified (tlc, mixture melting point, superimposable ir and nmr spectra) as **19** and **11**, respectively.

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References and Notes

(1) K. H. Lee, T. Ibuka, M. Kozuka, A. T. McPhail, and K. D. Onan, *Tetrahedron Lett.*, 2287 (1974) (part 10).
(2) K. H. Lee, *J. Pharm. Sci.*, **62**, 1028 (1973), and references cited therein.
(3) R. Adams and W. Herz, *J. Amer. Chem. Soc.*, **71**, 2551 (1949).
(4) K. H. Lee, H. Furukawa, and E. S. Huang, *J. Med. Chem.*, **15**, 609 (1972).
(5) R. Adams and W. Herz, *J. Amer. Chem. Soc.*, **71**, 2554 (1949).
(6) K. H. Lee, T. Ibuka, A. T. McPhail, K. D. Onan, T. A. Geissman, and T. G. Waddell, *Tetrahedron Lett.*, 1149 (1974).
(7) W. Herz, A. Romo de Vivar, J. Romo, and N. Viswanathan, *J. Amer. Chem. Soc.*, **85**, 19 (1963).
(8) K. H. Lee, H. Furukawa, S. H. Kim, and C. Piantadosi, *J. Pharm. Sci.*, **62**, 987 (1973).
(9) R. Adams and W. Herz, *J. Amer. Chem. Soc.*, **71**, 2546 (1949).
(10) K. H. Lee, R. Meck, C. Piantadosi, and E. S. Huang, *J. Med. Chem.*, **16**, 299 (1973).
(11) E. S. Huang, K. H. Lee, C. Piantadosi, T. A. Geissman, and J. S. Pagano, *J. Pharm. Sci.*, **61**, 1960 (1972).
(12) K. H. Lee and I. H. Hall, unpublished results.

Preparation and Antitumor Activity of a Rearranged Ester of Cephalotaxine†

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For further evaluation of structure–activity relationships among the *Cephalotaxus* alkaloids, a "rearranged" ester (**2b**) of cephalotaxine was prepared, one which is an isomer of deoxyharringtonine (**5a**). The parent alkaloid, cephalotaxine (**1a**), was allowed to react with thionyl chloride to replace its hydroxyl group with chlorine. The resulting chloro compound **1b**, on treatment with the silver salt of half ester **6**, yielded **2b** *via* an allylic rearrangement followed by further double bond migration. The new "rearranged" deoxyharringtonine isomer **2b** proved to be inactive in the P-388 lymphocytic leukemia system and thus further delineated the structural requirements for antitumor activity in this series of alkaloids.

A number of alkaloid esters from *Cephalotaxus harringtonia* have significant activity in experimental leukemia systems.[1,2] All of these active esters are derived from the same parent alkaloid, cephalotaxine (**1a**).[1,3,4] Although **1a** *per se* is inactive, there have been total syntheses of this unusual alkaloid by at least two groups,[5,6] as well as syntheses of the acyl moieties of three of its active esters—deoxyharringtonine (**5a**),[2,7a] harringtonine (**5b**),[7b] and isoharringtonine (**5d**).[7c] More recently, **5a** itself was prepared by partial synthesis from **1a**.[7d]

Variations in the acyl moiety of harringtonine and its active congeners naturally raise questions as to what structural features are essential for their antitumor activity. We demonstrated that mere introduction of an ester functionality does not transform **1a** into an active compound, since the acetate of **1a** is inactive (Table I). We also ascertained that the hindered *tert*-carboxyl group of the diacid moiety must be the one attached to cephalotaxine in order to preserve activity.[1,2] Accordingly, we have investigated another structural variation by synthesizing for bioassay purposes an isomer of deoxyharringtonine (**2b**) with the *tert*-carboxyl group joined to the alkaloid moiety but at a different position on the cyclopentene ring. The less accessible epimer **3** also was synthesized in small quantity.

Since the hydroxyl group of **1a** is in an allylic position, it seemed likely that we could replace it with chlorine and thus gain access to products of allylic rearrangement. This objective was realized by treating intermediate chloro compounds with the silver salt of the requisite half ester **6**. Rearranged esters were provided through a reaction that is formally SN2′-like in character, followed by a further double bond migration (see Scheme I).

When **1a** was treated with thionyl chloride in pyridine–ether solution, the main product was a chloro compound (**1b**) formed with inversion of configuration at C-3.‡ Reaction of **1b** with the silver salt of half ester **6** provided a new ester which was characterized as **2b** by nmr; its spectrum indicated that only one epimer was formed. Biological assay of "rearranged" ester **2b** revealed that its activity in the P-388 lymphocytic leukemia system, if any, is of a considerably lower order than that of harringtonine (**5b**), the

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‡Since the hydroxyl group of cephalotaxine has the *S* configuration,[8] the chloro compound that is derived by configurational inversion must have the *R* configuration. Accordingly, compound **1b** is (3*R*)-3-chloro-3-deoxycephalotaxine. For convenience, we call this compound epicephalotaxyl chloride and its epimer (**1d**) cephalotaxyl chloride. Asada[9] recently reported an analogous chloro compound prepared by treating **1a** with phosphorus oxychloride, but he did not assign a configuration to this derivative.