Studies on the Synthesis of Sesquiterpene Lactones, 12. Synthesis of (+)-Colartin, (+)-Arbusculin A and Their C-4 Epimers and Their Biological Activities

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STUDIES ON THE SYNTHESIS OF SESQUITERPENE LACTONES, 12.[1] SYNTHESIS OF (+)-COLARTIN, (+)-ARBUSCULIN A, AND THEIR C-4 EPIMERS AND THEIR BIOLOGICAL ACTIVITIES

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ABSTRACT.—Colartin [**9**] and arbusculin A [**11**] have been synthesized from α-santonin [**1**] in 14.5% (11 steps) and 9.3% (13 steps) overall yields, respectively. Arbusculin A [**11**] and compounds **20**, **21**, and **22**, which were derived from intermediate **2**, showed significant cell growth inhibitory activity against murine lymphocytic leukemia (P-388) in vitro. Plant growth regulating activity of **11** and its synthetic intermediates **4**, **5**, **8**, and **9** was also studied.

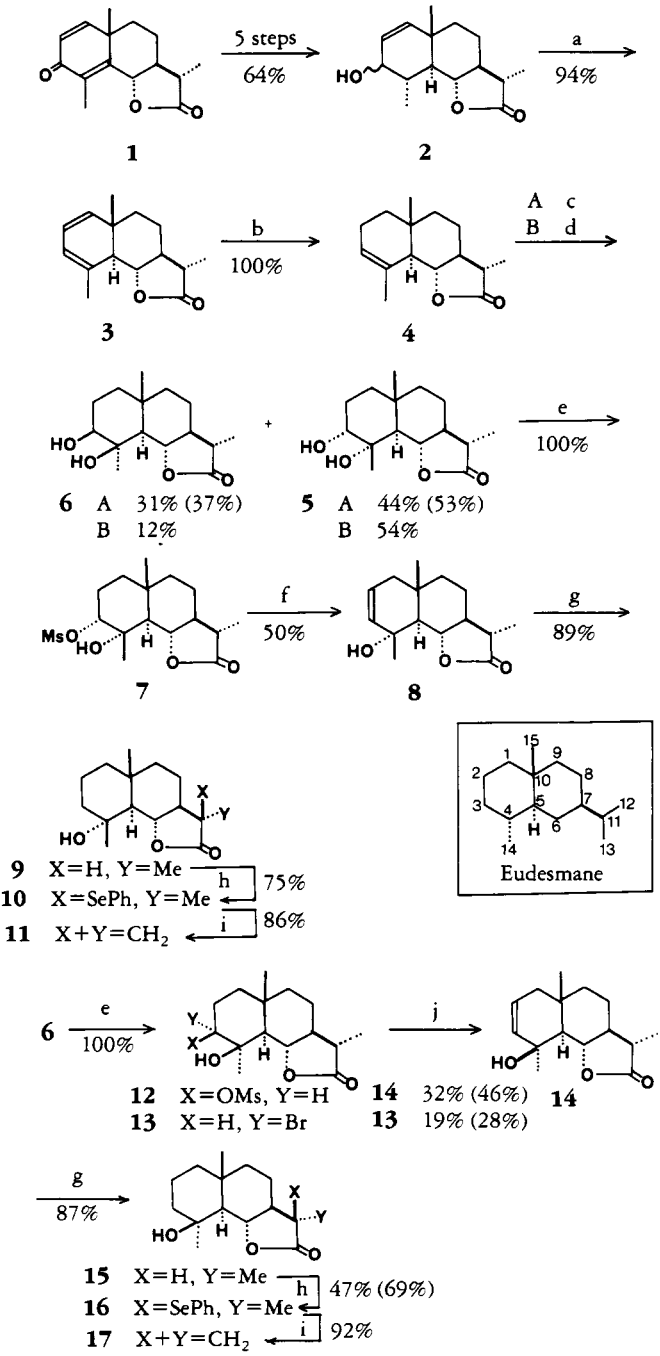
In the course of our program of study of the structure-activity relationship of α-methylene-γ-lactones (1), we encountered the necessity for efficient syntheses of (+)-colartin [**9**] (2), and (+)-arbusculin A [**11**] (2). Although their syntheses had already appeared in the literature (3), the reported methods were inconvenient for our purpose. In this paper we report efficient syntheses of (+)-colartin [**9**], (+)-arbusculin A [**11**], and their C-4 epimers **15** and **17**, along with their biological activities.

SYNTHESES OF COLARTIN, ARBUSCULIN A, AND THEIR C-4 EPIMERS.—The starting allylic alcohol **2** (4) was prepared from α-santonin [**1**] in 64% overall yield in 5 steps (Scheme 1). Treatment of **2** with boiling hexamethylphosphoric triamide (HMPA) (5) gave the known 1,3-diene **3** (4) in 94% yield. Selective hydrogenation of the C-1 double bond of **3** to give **4** was achieved by catalytic hydrogenation with Wilkinson's catalyst (6–8) in a quantitative yield. *cis*-Dihydroxylation of **4** with a catalytic amount of OsO_4 in the presence of *N*-methylmorpholine-*N*-oxide (NMO) (9) gave an α-diol **5** and a β-diol **6** in 53% and 37% yields, respectively. The preferential formation of **5** was observed when a stoichiometric amount of OsO_4 was employed in this transformation. The α and β orientations of the vicinal *cis*-hydroxyl groups of **5** and **6** were deduced from the following observations in their ^{1}H-nmr spectra as well as the reaction mechanism of OsO_4 with olefins. Thus, the 10-Me and H-6 resonances of **6** appeared at 0.16 and 0.11 ppm lower field than those of **5** due to the deshielding effect of the β(ax)-OH group at C-4 of **6** (10).

Mesylation of **5** with methanesulfonyl chloride and pyridine and successive treatment of the resulting mesylate **7** with a mixture of LiBr and Li_2CO_3 in DMF at 90° gave allylic alcohol **8** in 50% yield, accompanied by a 2:1 mixture of dienes **18** and **19**. Catalytic hydrogenation of **8** afforded a saturated tertiary α-alcohol **9** in 89% yield. Compound **9** was identical with colartin by comparison of their spectral data (2,3,11).

Phenylselenenylation of **9** and successive oxidative *syn*-elimination of the resulting phenylselenide **10** gave the desired α-methylene-γ-lactone **11** in 65% yield. Com-

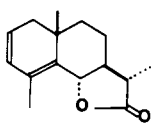
[1]For Part 11, see Ando *et al.* (1).



SCHEME 1. (a) HMPA, reflux, 16 min; (b) H_2, $(Ph_3P)_3RhCl$, EtOH-C_6H_6 (1:1); (c) OsO_4 (0.08 mol equiv), NMO (1.20 mol equiv), THF–H_2O–*t*-BuOH (230:25:1); (d) OsO_4 (1.1 mol equiv), THF-pyridine (4:1); then $NaHSO_3$, pyridine-H_2O (4:3); (e) MsCl, pyridine, room temperature; (f) LiBr, Li_2CO_3, DMF, 90°, 43 h; (g) H_2, Pt-C, EtOAc; (h) LDA, $(PhSe)_2$, THF, −78°, then −40°; (i) H_2O_2, HOAc-THF, 0°; (j) LiBr, Li_2CO_3, DMF, 115°, 83 h. Yields in parentheses are based on recovered starting materials.

pound **11** was identical with arbusculin A by comparison of their spectral data (2,3,11).

Conversion of the β-*cis* diol **6** to the C-4 epimers of colartin and arbusculin A was accomplished by a method analogous to that described above. Thus, mesylation of **6** and successive treatment of the resulting mesylate **12** with a mixture of LiBr and Li_2CO_3 in DMF at 115° for 83 h gave the desired allylic alcohol **14** and 3α-bromide **13** in 46% and 28% yields, respectively, accompanied by 10% yield of a 2:1 mixture of **18** and **19**. Attempted dehydrobromination of **13** using the same reaction conditions was unsuccessful and gave unchanged **13**. Catalytic hydrogenation of **14** gave the C-4 epimer **15** of colartin in 87% yield. Phenylselenenylation of **15** and successive treatment of the resulting phenylselenide **16** with H_2O_2 gave the C-4 epimer **17** of arbusculin A in 64% yield.



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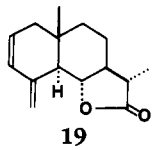

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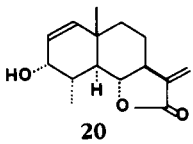

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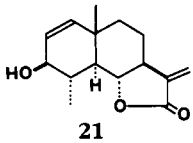

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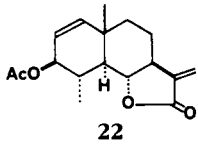

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BIOLOGICAL ACTIVITIES.—*Cell Growth Inhibitory Activity, P-388 Lymphocytic Leukemia.*—Arbusculin A [**11**] and compounds **20**, **21**, and **22**, which were derived from **2**, showed significant cell growth inhibitory activity against murine lymphocytic leukemia (P-388) in vitro. The results are summarized in Table 1.

Plant Growth Regulating Activity.—The plant growth regulating activity of compounds **4**, **5**, **8**, **9**, and **11** was studied employing three kinds of seeds, *Echinochloa frumentacea* (Japanese millet, Japanese name shokuyo hie), *Brassica juncea* (brown mustard, Japanese name seiyo karashina), and *Cucumis sativus* (cucumber, Japanese name kyuri). Germination of seeds and seedling growth were observed. The results are summarized in Table 2.

TABLE 1. Cell Growth Inhibitory Activity Against Murine Lymphocytic Leukemia (P-388) In Vitro.[a]

Compound	Concentration			
	10 g/ml	1 g/ml	10^{-1} g/ml	10^{-2} g/ml
11	101	45	2	
20	100	71	54	
21	100	48	24	
22	100	70	34	
Adriamycin (control)		100	91	33

[a]Cell growth inhibition ratio (%) = $1 - (T - C_O)/(C - C_O) \times 100$, where T = cell count after culture with compound, C = cell count after culture without compound, and C_O = cell count at the start of culture.

TABLE 2. Plant Growth Inhibitory Activities of Compounds **4**, **5**, **8**, **9**, and **11**.[a]

Compound	Plant	Part	Concentration					
			1000 ppm	500 ppm	250 ppm	100 ppm	50 ppm	10 ppm
4	*Echinochloa frumentacea*	stem	+	+	+	+	+	−
		root	+	−	−	−	−	−
	Brassica juncea	stem	−	−	−	−	−	−
		root	−	−	−	−	−	−
	Cucumis sativus	stem	+	+	−	−	−	−
		root	++	++	+	−	−	−
5	*E. frumentacea*	stem	++	++	+	+	+	−
		root	++	+	−	−	−	−
	B. juncea	stem	+	−	−	−	−	−
		root	++	+	−	−	−	−
	C. sativus	stem	+++	++	+	−	−	−
		root	+++	++	+	−	−	−
8	*E. frumentacea*	stem	++	+	+	+	+	−
		root	+++	++	+	−	−	−
	B. juncea	stem	++	+	−	−	−	−
		root	+++	++	−	−	−	−
	C. sativus	stem	+++	++	++	−	−	−
		root	+++	+++	++	−	−	−
9	*E. frumentacea*	stem	+++	++	+	+	−	−
		root	+++	++	+	−	−	−
	B. juncea	stem	+++	++	+	−	−	−
		root	+++	++	−	−	−	−
	C. sativus	stem	++	++	++	−	−	−
		root	+++	+++	++	+	−	−
11	*E. frumentacea*	stem	+++	++	−	−	−	−
		root	+++	++	+	−	−	−
	B. juncea	stem	+++	+++	++	−	−	−
		root	+++	+++	++	+	−	−
	C. sativus	stem	+++	+++	+	++	−	−
		root	+++	+++	++	++	−	−

[a]Key: +++, serious or complete inhibition of seed germination and seedling growth; ++, obvious effect on seed germination and seedling growth; +, slight effect; −, no effect.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All melting points are uncorrected. Ir spectra were recorded on a Hitachi 260-10 Spectrometer. ^{1}H-nmr spectra were recorded on a Varian EM-390 (90 MHz) spectrometer in $CDCl_3$. Coupling constants are in Hz. Mass spectra (eims) were recorded on a Hitachi M-52 spectrometer at 25 eV. Optical rotations were determined on a Perkin-Elmer 241 polarimeter in $CHCl_3$. Reactions were run under an atmosphere of N_2. THF was distilled from sodium benzophenone ketyl. Hexamethylphosphoric triamide (HMPA) and pyridine were distilled from CaH_2. DMF was dried by removing the C_6H_6-H_2O azeotrope. C_6H_6 was dried over sodium wire. Kiesel gel 60 (Merck 70–200 mesh) was employed for cc. Hplc was performed using a Kyowa-Seimitsu high quality pump (model KHP-011, KHD-W-294, KHD-W-600) equipped with a sample injection valve. The effluent was monitored with RI detectors (Shodex RI SE-11, Shodex RI SE-12). To describe hplc conditions, we designate column, solvent, flow rate (ml/min), and retention time (Rt) in minutes. The column codes are as follows: A, 250 × 4 mm i.d. stainless column packed with 10 μm Si gel (Kyowa gel MIC-SI-10); B, 250 × 8 mm i.d. stainless column packed with 10 μm Si gel (Kyowa gel MIC-SI-10); C, 300 × 20 mm i.d. stainless column packed with 15–25 μm Si gel (Merck, LiChroprep Si 60); D, 500 × 30 mm i.d. stainless column packed with 25–40 μm Si gel (Merck, LiChroprep si 60).

(11*S*)-3-HYDROXYEUDESM-1-ENO-13,6α-LACTONE [**2**].—The starting material **2** was prepared by the reported method (4) as a 4:1 mixture of β and α alcohols: mp 159–165°.

α-METHYLENE-γ-LACTONE DERIVATIVES OF **2**.—*3α-Hydroxyeudesma-1,11(12)-dieno-13,6α-lactone* [**20**].—Plates: mp 188°; hplc A, EtOAc-hexane (3:7), 3.2, Rt 7.2; ir ($CHCl_3$) 3610, 3500, 1765 cm^{-1}; ^{1}H nmr δ 0.95 (3H, s), 1.23 (3H, d, J = 6.0), 3.92 (1H, dd, J = 9.5, 9.5), 3.92 (1H, m), 5.36

(1H, d, J = 3.0), 5.67 (2H, m), 6.02 (1H, d, J = 3.0). *Anal.* calcd for $C_{15}H_{20}O_3$: C 72.55, H 8.12; found C 72.62, H 8.33.

3β-Hydroxyeudesma-1,11(12)-dieno-13,6α-lactone [**21**].—Fine needles: mp 128°; hplc A, EtOAc-hexane (3:7), 3.2, Rt 6.2; ir (KBr) 3240, 1768 cm^{-1}; ^1H nmr δ 1.05 (3H, s), 1.29 (3H, d, J = 6.0), 3.82 (1H, d, J = 8.2), 3.91 (1H, dd, J = 10.5, 10.5), 5.38 (1H, d, J = 3.2), 5.54 (2H, s), 6.06 (1H, d, J = 3.2). *Anal.* calcd for $C_{15}H_{20}O_3$: C 72.55, H 8.12; found C 72.77, H 8.33.

3β-Acetoxyeudesma-1,11(12)-dieno-13,6α-lactone [**22**].—Prisms: mp 128°; hplc A, EtOAc-hexane (3:7), 3.2, Rt 4.0; ^1H nmr δ 1.07 (3H, s), 1.15 (3H, d, J = 6.0), 2.06 (3H, s), 3.90 (1H, dd, J = 10.0, 10.0), 5.02 (1H, broad d, J = 8.0), 5.37 (1H, d, J = 3.4), 5.53 (2H, m), 6.04 (1H, d, J = 3.0). *Anal.* calcd for $C_{17}H_{22}O_4$: C 70.32, H 7.64; found C 70.09, H 7.90.

(11*S*)-EUDESMA-1,3-DIENO-13,6α-LACTONE [**3**].—A mixture of **2** (5.41 g, 22 mmol) and HMPA (50 ml) was refluxed (bath temperature 250°) for 15.5 min under vigorous stirring. The mixture was cooled, poured into 2 M aqueous solution of HCl (150 ml), and extracted with EtOAc (3 × 170 ml). Combined extracts were washed successively with 2 M HCl (30 ml), a saturated aqueous solution of $NaHCO_3$ (30 ml), and a saturated aqueous solution of NaCl (30 ml), dried (Na_2SO_4), and concentrated to give a colorless oil (8.0 g). This was then chromatographed over Si gel (80 g, 3.7 cm i.d. column) and eluted with EtOAc-hexane (1:15) to give 4.74 g (94%) of a mixture of **3** and (11*S*)-eudesma-2,4(5)-diene-13,6α-lactone [**18**] (a 15:1 mixture based on theanalyses of ^1H nmr and hplc) as colorless crystals (mp 86–88°). A part of this mixture was separated by hplc [A, EtOAc-hexane (1:9), 3]. The first peak (Rt 3.1) gave **18** as colorless crystals: mp 126°; ir (KBr) 1775 cm^{-1}; ^1H nmr δ 1.01 (3H, s), 1.25 (3H, d, J = 6.9), 1.96 (3H, d, J = 1.8), 4.50 (1H, broad d, J = 10.8), 5.69 (2H, m); eims *m/e* (rel. int.) $[M]^+$ 232 (100). The second peak (Rt 3.4) gave colorless plates, mp 96.5–98.5°, the spectral data of which were identical with those of **3** (4).

(11*S*)-EUDESM-3-ENO-13,6α-LACTONE [**4**].—The mixture of **3** (14.92 g, 64.2 mmol), EtOH (350 ml), C_6H_6 (350 ml), and $(Ph_3P)_3RhCl$ (1.19 g, 1.28 mmol) was shaken under 1 atm of H_2. H_2 uptake (1.4 liters) ceased after 3.5 h, and the mixture was filtered. The filtrate was concentrated under reduced pressure to give a brown solid which was chromatographed over Si gel (100 g, 3.7 cm i.d. column). The fraction eluted with EtOAc-hexane (1:10) (1 liter) gave a pale yellow solid (15.25 g, 100%), whose ^1H-nmr spectrum was identical with that of **4** (4,11).

CATALYTIC OSMYLATION OF **4**. FORMATION OF (11*S*)-3α,4α-DIHYDROXYEUDESMANO-13,6α-LACTONE [**5**] AND (11*S*)-3β,4β-DIHYDROXYEUDESMANO-13,6α-LACTONE [**6**].—The mixture of **4** (8.417 g, 35.9 mmol), *N*-methylmorpholine-*N*-oxide (NMO, 5.04 g, 43.1 mmol), THF (168 ml), H_2O (18.3 ml), and 3.93 M *t*-BuOH solution of OsO_4 (0.73 ml, 2.87 mmol) was stirred at room temperature for 20 h. A slurry of $Na_2S_2O_4$ (9.0 g), talc (59 g), and H_2O (210 ml) was added, stirred vigorously for 30 min, and filtered through celite. The filtrate was extracted with EtOAc (200 ml, 2 × 100 ml). Combined extracts were washed successively with a saturated aqueous solution of NaCl, dried, and concentrated to give a yellow oil, which was chromatographed over Si gel (60 g, 3.5 cm i.d. column) and eluted with EtOAc-hexane (3:7).

The first fraction (180 ml) gave recovered **4** (1.378 g, 16%). The second fraction gave a mixture of **5** and **6** (7.775 g), which was separated by hplc [D, EtOAc-hexane (4:6), 30].

The first peak (Rt 28) gave **5** (4.395 g, 44%) as colorless needles: mp 179–180°; ir (KBr) 3580, 3540, 1780 cm^{-1}; ^1H nmr δ 1.01 (3H, s), 1.28 (3H, d, J = 6.8), 1.31 (3H, s), 2.07 (1H, d, J = 11.8), 2.26 (1H, m), 3.62 (1H, t, J = 2.3), 4.07 (1H, dd, J = 12.7, 11.8); $[\alpha]^{24}D$ −5.8° (c = 1.09). *Anal.* calcd for $C_{15}H_{24}O_4$: C 67.14, H 9.01; found C 66.96, H 9.35.

The second peak (Rt 39) gave **6** (3.273 g, 31%) as colorless needles: mp 149°; ir (KBr) 3515, 3420, 1780 cm^{-1}; ^1H nmr δ 1.17 (3H, s), 1.21 (3H, d, J = 7.4), 1.48 (3H, s), 3.25 (1H, t, J = 8.3), 4.18 (1H, dd, J = 10.5, 9.0); $[\alpha]^{24}D$ +45.7° (c = 1.06). *Anal.* calcd for $C_{15}H_{24}O_4$: C 67.17, H 9.01; found C 67.03, H 9.23.

OSMYLATION OF **4** WITH A MOLAR EQUIVALENT OF OsO_4.—To a solution of **4** (118.4 mg, 0.504 mmol) in a mixture of THF (4 ml) and pyridine (1 ml) was added OsO_4 (141 mg, 0.554 mmol). The mixture was stirred for 5 h at room temperature and concentrated. The residue (2 ml) was stirred for 1 h with a solution of $NaHSO_3$ (0.4 g) in a mixture of pyridine (7 ml) and H_2O (6 ml). The orange solution was extracted with $CHCl_3$ (30 ml, 2 × 20 ml). Combined extracts were washed with saturated aqueous NaCl (2 × 20 ml), dried (Na_2SO_4), and concentrated to give an oily material (140 mg), which was chromatographed over Si gel (4 g, 1.2 cm i.d. column) and eluted with EtOAc-hexane (1:2) to give an oily material (120 mg). This was purified by hplc [B, EtOAc-hexane (3:7), 7.5]. The first peak (Rt 7.6) gave **5** (72.9 mg, 54%). The second peak (Rt 11.2) gave **6** (16 mg, 12%).

(11*S*)-4α-HYDROXY-3α-(MESYLOXY)EUDESMANO-13,6α-LACTONE [**7**].—To a stirred solution of **5** (801.1 mg, 3.00 mmol) in pyridine (28 ml) was added methanesulfonyl chloride (0.58 ml, 7.5 mmol). The mixture was stirred for 12 h at room temperature, poured into a saturated aqueous solution of NaCl (100 ml), and extracted with EtOAc (2 × 250 ml, 100 ml). The combined extracts were washed successively with 2 M aqueous HCl (50 ml), saturated aqueous $NaHCO_3$ (50 ml), and saturated aqueous NaCl (50 ml), dried (Na_2SO_4), and concentrated to give an oil (1.5 g), which was chromatographed over Si gel (20 g, 2 cm i.d. column) and eluted with EtOAc-hexane (2:3) to give **7** (1.0896 g, 100%) as a colorless oil: ir (KBr) 3570, 1775 cm^{-1}; ^1H nmr δ 1.04 (3H, s), 1.25 (3H, d, J = 6.6), 1.37 (3H, s), 2.33 (1H, m), 3.09 (3H, s), 4.05 (1H, dd, J = 11.9, 9.6), 4.56 (1H, dd, J = 3.2, 3.2); $[\alpha]^{24}D$ −28.8° (c = 1.12). *Anal.* calcd for $C_{16}H_{26}O_6S$: C 55.47, H 7.56, S 9.25; found C 55.31, H 7.40, S 9.34.

(11*S*)-4α-HYDROXYEUDESM-2-ENO-13,6α-LACTONE [**8**].—A mixture of **7** (73.5 mg, 0.213 mmol), Li_2CO_3 (47 mg, 0.639 mmol), and LiBr (40 mg, 0.426 mmol) in anhydrous DMF (2.5 ml) was stirred at 90° for 43 h, cooled, poured into saturated aqueous solution of NaCl (20 ml), and extracted with EtOAc (50 ml, 2 × 20 ml). The combined extracts were washed successively with a 2 M aqueous solution of HCl (2 × 10 ml) and saturated aqueous NaCl (20 ml), dried ($MgSO_4$), and concentrated to give an oily material (54.8 mg), which was chromatographed over Si gel (3 g, 1.2 cm i.d. column) and eluted with EtOAc-hexane (1:4).

The first fraction (30 ml) gave a 2:1 mixture of **18** and (11*S*)-eudesma-2,4(14)-dieno-13,6α-lactone [**19**] as a crystalline material (7.4 mg, 16%). This fraction was further purified by hplc [A, EtOAc-hexane (5:95), 3]. The first peak (Rt 5.2) gave **18** (4.5 mg). The second peak (Rt 7.2) gave **19** (2.5 mg) as colorless needles: mp 136°; ir (KBr) 1770 cm^{-1}; ^1H nmr δ 0.86 (3H, s), 1.24 (3H, d, J = 6.5), 2.37 (1H, broad d, J = 10.6), 3.97 (1H, dd, J = 10.6, 7.2), 5.00 (1H, broad s), 5.28 (1H, broad s), 5.63 (1H, m), 6.08 (1H, broad d); eims *m/z* (rel. int.) $[M]^+$ 232 (90), 216 (100). The second fraction (30 ml) gave **8** (26.5 mg, 50%) as colorless prisms: mp 122.5°; ir (KBr) 3580, 1775, 1660 cm^{-1}; ^1H nmr δ 1.02 (3H, s), 1.26 (3H, d, J = 6.8), 1.39 (3H, s), 2.33 (1H, m), 4.17 (1H, dd, J = 11.4, 9.2); $[\alpha]^{24}D$ +78.7° (c = 0.79). *Anal.* calcd for $C_{15}H_{22}O_3$: C 71.96, H 8.86; found C 71.73, H 8.62.

COLARTIN [**9**].—A mixture of **8** (1.26 g, 5.033 mmol), EtOAc (150 ml), PtO_2 (23 mg), and activated charcoal powder (128 mg) was shaken under 1 atm of H_2. H_2 uptake (110 ml) ceased after 1 h and the mixture was filtered. The filtrate was concentrated to give spectroscopically pure **9** (1.29 g, 89%) as a white solid, which was subsequently recrystallized from a mixture of hexane and Et_2O (1:1) to give colorless plates: mp 109–110°; ir (KBr) 3580, 1780 cm^{-1}; ^1H nmr δ 1.00 (3H, s), 1.20 (3H, d, J = 6.8), 1.33 (3H, s), 1.72 (1H, d, J = 11.4), 4.02 (1H, dd, J = 11.4, 9.0); $[\alpha]^{20}D$ +11.4° (c = 0.97). *Anal.* calcd for $C_{15}H_{24}O_3$: C 71.39, H 9.59; found C 71.12, H 9.43.

4α-HYDROXY-11β-(PHENYLSELENO)EUDESMANO-13,6α-LACTONE [**10**].—To a THF solution of lithium diisopropylamide (11.79 mmol) [prepared from diisopropylamine (1.65 ml), 1.6 M butyllithium in hexane (7.37 ml), and THF (35 ml) at −75°] 1.19 g (4.716 mmol) of **9** in THF (10 ml) was added dropwise over a period of 10 min. After the solution was stirred at −78° for 1 h, diphenyl diselenide (3.68 g, 11.79 mmol) in THF (15 ml) containing HMPA (2.05 ml, 11.79 mmol) was added dropwise at −78° over a period of 20 min. The reaction mixture was stirred at −78° for 40 min and then warmed to −40°, where stirring was continued for an additional 35 min. The reaction was quenched by the addition of a mixture of 2 M aqueous HCl and saturated aqueous NaCl (1:1, 60 ml) and extracted with EtOAc (3 × 50 ml). The combined extracts were washed successively with saturated aqueous $NaHCO_3$ (20 ml) and saturated aqueous NaCl (20 ml), dried ($MgSO_4$), and concentrated to give a yellow oil (5.0 g), which was chromatographed over Si gel (100 g, 3.7 cm i.d. column). The fraction eluted with hexane gave diphenyl diselenide. The fraction eluted with EtOAc-hexane (3:7) (1 liter) gave spectroscopically pure **10** (1.434 g, 75%). The analytical sample was obtained by recrystallization from EtOAc-hexane (1:1) as colorless needles: mp 154.5–157.5°; ir (KBr) 3580, 1775 cm^{-1}; ^1H nmr δ 0.98 (3H, s), 1.26 (3H, s), 1.55 (3H, s), 2.99 (1H, broad s, -OH), 4.36 (1H, dd, J = 11.4, 9.9), 7.55 (5H, m); $[\alpha]^{24}D$ +59.0 (c = 1.23). *Anal.* calcd for $C_{21}H_{28}O_3Se$: C 61.91, H 6.93; found C 61.72, H 7.20.

ARBUSCULIN A [**11**].—A solution of **10** (1.0369 g, 2.55 mmol) in THF (15.6 ml) containing HOAc (0.37 ml) was treated at 0° with 30% H_2O_2 (2.3 ml) for 1 h. The reaction mixture was poured into cold saturated aqueous $NaHCO_3$ (50 ml) and extracted with EtOAc (3 × 50 ml). The combined extracts were washed successively with 10% aqueous $Na_2S_2O_3$ (20 ml) and saturated aqueous NaCl (20 ml), dried (Na_2SO_4), and concentrated to give a yellow oil (1.5 g), which was chromatographed over Si gel (40 g, 3.5 cm i.d. column) and eluted with EtOAc-hexane (2:8) (560 ml) to give **11** (546.3 mg, 86%) as colorless crystalline material. The analytical sample of **11** was obtained by the recrystallization from hexane-Et_2O (1:1) as colorless plates: mp 73°; ir (KBr) 3590, 1765, 1670 cm^{-1}; ^1H nmr δ 0.99 (3H, s), 1.34 (3H, s), 1.83 (1H, d, J = 11.4), 2.57 (1H, m), 4.01 (1H, dd, J = 11.4, 10.7), 5.40 (1H, d, J = 3.0), 6.06 (1H,

d, $J=3.2$); $[\alpha]^{20}D$ +25.8° ($c=1.33$). *Anal.* calcd for $C_{15}H_{22}O_3$: C 71.97, H 8.86; found C 72.14, H 8.83.

(11*S*)-4β-HYDROXY-3β-(MESYLOXY)EUDESMANO-13,6α-LACTONE [**12**].—To a stirred solution of **6** (74 mg, 0.277 mmol) in pyridine (3 ml) was added methanesulfonyl chloride (32 μl, 0.032 mmol). The mixture was stirred for 12 h at room temperature, poured into saturated aqueous NaCl (15 ml), and extracted with EtOAc (3 × 30 ml). The combined extracts were washed successively with 2 M aqueous HCl (2 × 15 ml), saturated aqueous $NaHCO_3$ (15 ml), and saturated aqueous NaCl (15 ml), dried ($MgSO_4$), and concentrated to give spectroscopically pure **12** (102.3 mg, 100%) as a crystalline material. The analytical sample was obtained by recrystallization from EtOAc-hexane (1:1) as colorless prisms: mp 182°; ir (KBr) 3545, 1760 cm^{-1}; ^1H nmr δ 1.20 (3H, s), 1.22 (3H, d, $J=7.5$), 1.53 (3H, s), 3.07 (3H, s), 4.21 (1H, dd, $J=10.5$, 8.1), 4.37 (1H, dd, $J=11.6$, 5.7); $[\alpha]^{24}D$ +33.1° ($c=1.57$). *Anal.* calcd for $C_{16}H_{26}O_6S$: C 55.47, H 7.56, S 9.25; found C 55.68, H 7.68, S 9.42.

(11*S*)-4β-HYDROXYEUDESMAN-2-ENO-13,6α-LACTONE [**14**].—A mixture of **12** (2.0304 g, 5.88 mmol), Li_2CO_3 (977.6 mg, 12.23 mmol), and LiBr (765.9 mg, 8.82 mmol) in anhydrous DMF (55 ml) was stirred at 111–115° for 83 h, cooled, poured into a mixture of 2 M aqueous HCl (40 ml) and saturated aqueous solution of NaCl (500 ml), and extracted with EtOAc (200 ml, 2 × 150 ml). The combined extracts were washed successively with saturated aqueous $NaHCO_3$ (50 ml) and saturated aqueous NaCl (50 ml), dried (Na_2SO_4), and concentrated to give an oily material, which was chromatographed over Si gel (10 g, 3.0 cm i.d. column) and eluted with EtOAc. The eluate was concentrated and further purified by hplc [D, EtOAc-hexane (3:7), 44]. The first peak (Rt 8) gave a 2:1 mixture of dienes **18** and **19** as a crystalline material (99.8 mg, 7%). The second peak (Rt 12) gave (11*S*)-3α-bromo-4β-hydroxyeudesmano-13,6α-lactone [**13**] as a spectroscopically pure solid (378.7 mg, 19%). The analytical sample of **13** was obtained by recrystallization from EtOAc-hexane (1:1) as colorless prisms: mp 167°; ir (KBr) 3500, 1755 cm^{-1}; ^1H nmr δ 1.16 (3H, s), 1.21 (3H, d, $J=6.3$), 1.56 (3H, s), 1.84 (1H, d, $J=11.4$), 2.33 (2H, m), 3.88 (1H, dd, $J=3.0$, 3.0), 4.13 (1H, dd, $J=11.4$, 9.2); eims *m/e* (rel. int.) $[M]^+$ (^{81}Br) 332 (0.93), $[M]^+$ (^{79}Br) 330 (1.56), 208 (100).

The third peak (Rt 16) gave spectroscopically pure **14** as a colorless crystalline solid (468.2 mg, 32%). The analytical sample of **14** was obtained by recrystallization from EtOAc as colorless prisms: mp 123°; ir (KBr) 3430, 1785 cm^{-1}; ^1H nmr δ 1.10 (3H, s), 1.25 (3H, d, $J=6.8$), 1.44 (3H, s), 2.28 (1H, m), 4.24 (1H, dd, $J=11.4$, 10.5), 5.62 (2H, m); $[\alpha]^{20}D$ +83.6° ($c=0.89$). *Anal.* calcd for $C_{15}H_{22}O_3$: C 71.97, H 8.86; found C 71.74, H 8.85.

The fourth peak (Rt 28) gave recovered **12** (632 mg, 30%).

(11*S*)-4β-HYDROXYEUDESMANO-13,6α-LACTONE [**15**].—A mixture of **14** (28 mg, 0.112 mmol), EtOAc (4 ml), PtO_2 (2.6 mg), and activated charcoal powder (28 mg) was shaken under 1 atm of H_2. H_2 uptake (10 ml) ceased after 30 min, and the mixture was filtered through celite. The filtrate was concentrated to give an oil (25 mg), which was chromatographed over Si gel [1 g, 1.2 cm i.d. column, EtOAc-hexane (1:3)]. The first fraction (60 ml) gave spectroscopically pure **15** (24.6 mg, 87%) as a colorless crystalline material, which was recrystallized from a mixture of hexane and EtOAc to give colorless prisms: mp 115°; ir (KBr) 3550, 1767 cm^{-1}; ^1H nmr δ 1.16 (3H, s), 1.20 (3H, d, $J=6.3$), 1.40 (3H, s), 2.25 (1H, m), 4.13 (1H, dd, $J=10.8$, 9.3); $[\alpha]^{24}D$ +41.6° ($c=1.16$). *Anal.* calcd for $C_{15}H_{24}O_3$: C 71.39, H 9.59; found C 71.05, H 9.71.

4β-HYDROXY-11β-(PHENYLSELENO)EUDESMANO-13,6α-LACTONE [**16**].—To a THF solution of lithium diisopropyl amide [prepared from diisopropylamine (0.59 ml, 4.195 mmol), 1.60 M butyllithium in hexane (2.62 ml, 4.195 mmol), and THF (13 ml) at −78°] was added dropwise over a period of 10 min 423 mg (1.678 mmol) of **15** in THF (5 ml). After the solution was stirred at −78° for 1 h, diphenyl diselenide (1.309 g, 4.195 mmol) in THF (5 ml) containing HMPA (0.73 ml, 4.195 mmol) was added dropwise at −78° over a period of 15 min. The reaction mixture was stirred at −78° for 1 h and then warmed at −40°, where stirring was continued for an additional 30 min. The reaction mixture was quenched by addition of a mixture of 0.2 M aqueous HCl (30 ml) and NaCl (5 g). The mixture was extracted with EtOAc (100 ml, 2 × 50 ml). The combined extracts were washed successively with saturated aqueous $NaHCO_3$ (30 ml) and saturated aqueous NaCl (30 ml), dried (Na_2SO_4), and concentrated to give crude product (2.2 g) as a yellow oil, which was subsequently chromatographed over Si gel (50 g, 2.7 cm i.d. column). After elution with hexane (700 ml), the fraction eluted with EtOAc-hexane (1:2) (200 ml) gave an oily mixture (704.5 mg). This was further purified by hplc [C, EtOAc-hexane (2:8), 30]. The first peak (Rt 4) gave **16** (324 mg, 47%) as colorless prisms: mp 136°; ir (KBr) 3530, 1755 cm^{-1}; ^1H nmr δ 1.22 (3H, s), 1.43 (3H, s), 1.55 (3H, s), 4.64 (1H, dd, $J=11.7$, 9.0), 7.51 (5H, m). *Anal.* calcd for $C_{21}H_{28}O_3Se$: C 61.91, H 6.93; found C 61.98, H 6.80. The second peak (Rt 8.8) gave recovered **15** (133 mg, 32%).

4β-HYDROXYEUDESM-11(12)-ENO-13,6α-LACTONE [**17**].—A solution of **16** (35.3 mg, 0.0866

mmol) in THF (1 ml) containing HOAc (12.4 liters, 0.216 mmol) was treated at 0° with 30% H_2O_2 (0.1 ml, 0.868 mmol) for 30 min. The reaction mixture was poured into a mixture of cold saturated aqueous $NaHCO_3$ (5 ml) and NaCl (1 g) and extracted with EtOAc (30 ml, 2 × 10 ml). Combined extracts were washed with saturated aqueous NaCl (5 ml), dried (Na_2SO_4), and concentrated to give 28 mg of a crude crystalline product. This was then purified by hplc [B, EtOAc-hexane (2:8), 7.5, Rt 4] to give **17** (20 mg, 92%) as colorless needles: mp 150.5°; ir (KBr) 3480, 1765 cm^{-1}; ^1H nmr δ 1.16 (3H, s), 1.43 (3H, s), 1.47 (1H, d, J = 10.7), 2.50 (1H, m), 4.12 (1H, dd, J = 10.7, 10.4), 5.43 (1H, d, J = 3.0), 6.20 (1H, d, J = 3.2); $[\alpha]^{24}D$ +51.9° (c = 0.79). *Anal.* calcd for $C_{15}H_{22}O_3$: C 71.97, H 8.86; found C 72.23, H 9.01.

THE CELL GROWTH INHIBITORY ACTIVITY OF COMPOUNDS TO MURINE LYMPHOCYTIC LEUKEMIA CELL (P-388) IN VITRO.—Murine lymphocytic leukemia cells (P-388) were incubated with the compounds at 37° in a humidified atmosphere of 5% CO_2 for 48 h. After incubation, cells were counted with a Coulter counter (Model ZBI, Coulter Electronics, Hialeah, Florida), and the cell growth inhibition ratio (%) was calculated (Table 1).

THE PLANT GROWTH REGULATING ACTIVITY OF COMPOUNDS.—Compounds (15 mg) were dissolved in 0.3 ml of solvent [Me_2CO-Tween 80 (10:1)] and diluted with H_2O to give test solutions. Three kinds of seeds, *Echinochloa frumentaceae, Brassica juncea,* and *Cucumis sativus*, were sown in a Petri dish (10 cm × 15 cm) containing 10 ml of the test solution and incubated under light (4000 lux) at 27° for 10 days. Germination of seeds and growth of seedlings were observed and examined (Table 2).

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