Studies on the Synthesis of Sesquiterpene Lactones, 14. Syntheses of (-)-Arbusclin D and (+)-4-epi-Arbusclin D: The Stereochemical Assignment of Arbusclin D

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J. Nat. Prod., **1992**, 55 (11), 1620-1625• DOI: 10.1021/np50089a009 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 55, No. 11, pp. 1620-1625, November 1992

STUDIES ON THE SYNTHESIS OF SESQUITERPENE LACTONES, 14.[1] SYNTHESES OF (−)-ARBUSCLIN D AND (+)-4-*EPI*-ARBUSCLIN D: THE STEREOCHEMICAL ASSIGNMENT OF ARBUSCLIN D

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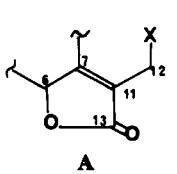
ABSTRACT.—Efficient syntheses of (−)-arbusclin D and (+)-4-*epi*-arbusclin D are reported. By these syntheses the C-4 stereochemistry of arbusclin D and the absolute configuration of (−)-arbusclin D have been determined to be a s shown in structure **1**. The biological activities, such as cytotoxic activity toward P-388 lymphocytic leukemia, plant growth regulating activity, and antimicrobial activity of compounds **1**, **3**, **7**, **9**, **12**, and **14** were also studied.

In a previous paper (1) we reported on an efficient methodology for the synthesis of C-12 functionalized endocyclic α,β-unsaturated γ-lactones such as **A** (X=OH, OAc, Br) (2). In the present paper we present an application of this methodology to the synthesis of arbusclin D, a representative naturally occurring sesquiterpene of this type.

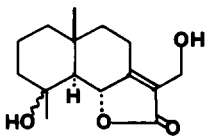
The structure of arbusclin D was proposed as **B** by Irwin and Geissman (3) on the basis of spectral data, but spectral and chemical evidence for the C-4 stereochemistry and the absolute configuration of this compound were not established. We decided therefore to synthesize the two possible diastereoisomers of arbusclin D, 4α,12-dihydroxyeudesm-7(11)-eno-13,6α-lactone [**1**] and 4β,12-dihydroxyeudesm-7(11)-eno-13,6α-lactone [**2**], in optically active form.

The starting material (+)-arbusclin A [**3**] and (+)-4-*epi*-arbusclin A [**4**] (Scheme 1) with known stereochemistry (4) were synthesized from santonin (5,6). Bromination of **3** with bromine in CH_2Cl_2, in the presence of NaOAc, gave dibromide **5** stereoselectively in 81% yield. The paramagnetic shift of H-6 of **5** (0.37 ppm) compared with that of **12** (4–6) indicates the β orientation of bromine atoms at C-11.

Dehydrobromination of **5** with a mixture of LiBr and Li_2CO_3 in DMF at 75° gave a C-12 brominated endocyclic α,β-unsaturated γ-lactone derivative **7** in 84% yield.

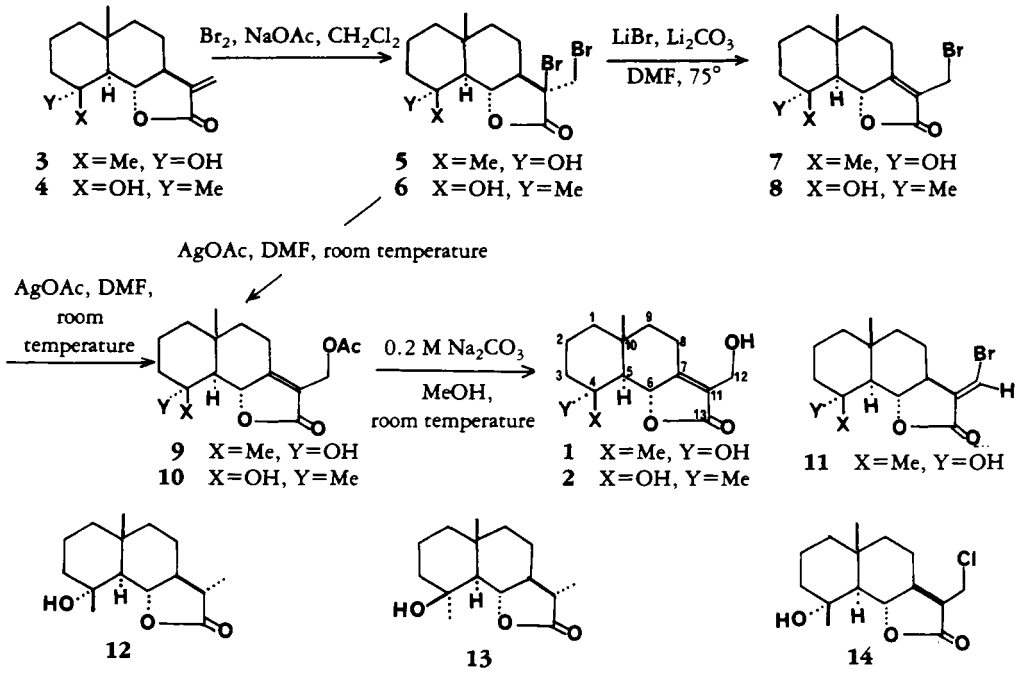


A



B

[1]A preliminary report of this work was presented at the 31st Symposium on the Chemistry of Terpenes, Essential Oils, and Aromatics (TEAC), Kyoto, September, 1987. For Part 13, see Ando *et al.* (1).



SCHEME 1

Treatment of **7** with AgOAc in DMF at room temperature gave an acetate **9** in 74% yield. Alternatively, treatment of **5** with AgOAc in DMF at room temperature gave **9** directly, in 69% yield, accompanied by exocyclic α,β-unsaturated γ-lactone [**11**] (8%). Cleavage of **9** with a 0.2 M aqueous solution of Na_2CO_3 in MeOH at room temperature gave 4α,12-dihydroxyeudesm-7(11)-eno-13,6α-lactone [**1**] in 63% yield.

Conversion of (+)-4-*epi*-arbusclin A [**4**] to **2**, the C-4 epimer of **1**, was accomplished by a method analogous to that described above. Thus, bromination of **4** with bromine in CH_2Cl_2 in the presence of NaOAc gave dibromide **6** stereoselectively in 78% yield. The paramagnetic shift of H-6 of **6** (0.39 ppm) compared with that of **13** (5) indicates the β orientation of bromine atoms at C-11.

Dehydrobromination of **6** with a mixture of LiBr and Li_2CO_3 in DMF at 75° gave a C-12 brominated endocyclic α,β-unsaturated γ-lactone derivative **8** in 78% yield. Treatment of **8** with AgOAc in DMF at room temperature gave an acetate **10** in 24% yield. Under the same reaction conditions **6** gave **10** in 34% yield. Cleavage of **10** with 0.2 **M** aqueous solution of Na_2CO_3 in MeOH at room temperature gave 4β,12-dihydroxyeudesm-7(11)-eno-13,6α-lactone [**2**] in 73% yield.

Compound **1** was identical with arbusclin D by comparison of their ^{1}H-nmr spectral data and melting points (3). Compound **2** showed a significantly different ^{1}H-nmr spectrum and melting point compared with those of arbusclin D. Since the $[\alpha]_D$ value of naturally occurring arbusclin D was not reported, we could not determine its absolute configuration. However, the absolute configuration of (−)-arbusclin D was determined to be as shown in structure **1** by this synthesis.

To examine the relationship between sesquiterpene structure and cytotoxic, plant growth regulating, and antimicrobial activities, the compounds **1**, **3**, **7**, **9**, **12**, and **14** were assayed. Because of the very limited amount of **4** available and the poor yield of **10**, we could not test the compounds of the 4β-OH series, **2**, **4**, **6**, **8**, **10**, and **13**.

The α-methylene-γ-lactone **3**, C-12 brominated compound **7**, and C-12 chlori-

nated compound **14** showed significant cell growth inhibitory activity against murine lymphocytic leukemia (P-388) in vitro (Table 1). It is interesting that C-12 functionalized endocyclic α,β-unsaturated γ-lactone derivatives **7** and **14** showed significant activity as well as α-methylene γ-lactone derivative **3**.

The plant growth regulating activity of compounds **1**, **3**, **7**, **9**, **12**, and **14** was studied using 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. It is noteworthy that the compounds **3** and **14** showed significant growth inhibitory activity toward *C. sativus* at 100 ppm.

TABLE 1. Cell Growth Inhibitory Activity Against Murine Lymphocytic Leukemia (P-388) in Vitro.

Compound	Cell growth inhibitory ratio,[a] %			
	10 μg/ml	1 μg/ml	10^{-1} μg/ml	10^{-2} μg/ml
3	101	45	2	
7	102	44	3	
14	103	65	26	
adriamycin (control)		105	99	38

[a]Cell growth inhibitory ratio (%) $= (1 - \frac{T-C_O}{C-C_O}) \times 100$ where T = cell count after the culture in the presence of the compounds, C = cell count after the culture in the absence of the compounds, and C_O = cell count at the start of the culture.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All melting points are uncorrected. ^{1}H-nmr spectra were recorded at 90 MHz and 200 MHz in $CDCl_3$. Coupling constants are in Hz. Mass spectra (eims) were recorded at 25 eV. Optical rotations were determined in $CHCl_3$. Reactions were run under an atmosphere of N_2. DMF was dried by removing the C_6H_6/H_2O azeotrope. CH_2Cl_2 was distilled from CaH_2. Kieselgel 60 (Merck 70–200 mesh) was employed for cc. To describe hplc conditions, we designate column, solvent, flow rate in ml/min, and the retention time (Rt) in minutes in this order. The column codes are as follows: A, 250 × 4 mm i.d. stainless steel column packed with 10 μm Si gel; B, 250 × 8 mm i.d. stainless steel column packed with 10 μm Si gel; C, 300 × 20 mm i.d. stainless steel column packed with 15–25 μm Si gel.

11β,12-DIBROMO-4α-HYDROXYEUDESMANO-13,6α-LACTONE [**5**].—To a stirred mixture of arbusclin A [**3**] (534 mg, 2.13 mmol) and NaOAc (262 mg, 3.2 mmol) in CH_2Cl_2 (25 ml) was added a solution of Br_2 (120 μl, 2.34 mmol) in CH_2Cl_2 (0.66 ml) at 0°. The mixture was stirred for 15 min at 0° and for 4 h at room temperature and poured into saturated aqueous $NaHCO_3$ (20 ml). The organic layer was separated, and the aqueous layer was extracted with $CHCl_3$ (3 × 50 ml). The combined organic layers 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 pale yellow crystalline material, which was separated by hplc [C, EtOAc-hexane (2:8), 29].

The first peak (Rt 6.6) gave spectroscopically pure **5** (705 mg, 81%) as a white solid, which was crystallized from a mixture of hexane and EtOAc to give colorless needles: mp 113°; ir (KBr) 3595, 1785 cm^{-1}; ^{1}H nmr (90 MHz) δ 1.02 (3H, s, 10-Me), 1.36 (3H, s, 4-Me), 1.86 (1H, d, J = 11.7, H-5), 3.82 (1H, d, J = 10.7, H-12), 4.13 (1H, d, J = 10.7, H-12), 4.39 (1H, dd, J = 11.7, 9.6, H-6); $[\alpha]^{24}D$ +22.0 (c = 1.04). *Anal.* calcd for $C_{15}H_{22}O_3Br_2$, C 43.93, H 5.41, Br 38.96; found C 43.69, H 5.47, Br 39.08.

TABLE 2. Plant Growth Inhibitory Activities of Compounds **1**, **3**, **7**, **9**, **12**, and **14**.[a]

Compound	Plant	Part	Activities (ppm)				
			1000	500	250	100	50
1	*Echinochloa frumentacea*	stem	++	+	−		
		root	++	−	−		
	Brassica juncea	stem	+	+	+		
		root	++	−	−		
	Cucumis sativus	stem	−	−	−		
		root	+++	++	++		
3	*E. frumentacea*	stem	+++	+++	+	+	−
		root	+++	+++	+	−	−
	B. juncea	stem	+++	+++	++	−	−
		root	+++	+++	+++	−	−
	C. sativus	stem	+++	+++	++	++	−
		root	+++	+++	++	++	−
7	*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	+++	++	+		
12	*E. frumentacea*	stem	+++	++	+	+	−
		root	+++	+++	+	−	
	B. juncea	stem	+++	++	+	−	−
		root	+++	+++	−	−	
	C. sativus	stem	+++	+++	+++	−	−
		root	+++	+++	+++	+	−
14	*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 grmination and seedling growth, +, slight effect; −, no effect.

12-BROMO-4α-HYDROXYEUDESM-7(11)-ENO-13,6α-LACTONE [**7**].—A mixture of **5** (494 mg, 1.20 mmol), Li_2CO_3 (222 mg, 3.01 mmol), and LiBr (170 mg, 11.9 mmol) in DMF (22 ml) was stirred at 75° for 40 min, cooled to room temperature, poured into 1 M aqueous H_2SO_4 (20 ml), and extracted with EtOAc (3 × 50 ml). The combined extracts were washed successively with saturated aqueous $NaHCO_3$ (20 ml) and saturated aqueous NaBr (50 ml), dried ($MgSO_4$), and concentrated to give a pale yellow oil (1.0 g), which was chromatographed over Si gel (25 g, 1.5 cm i.d. column) and eluted with hexane-EtOAc (7:3) to give spectroscopically pure **7** (333 mg, 84%) as a colorless solid. Recrystallization from a mixture of hexane and Et_2O gave colorless prisms: mp 103.5°; ir (KBr) 3570, 1770, 1675 cm^{-1}; ^1H nmr (90 MHz) δ 1.08 (3H, s, 10-Me), 1.40 (3H, s, 4-Me), 1.47 (1H, d, J = 11.6, H-5), 2.55 (1H, ddd, J = 14.8, 12.9, 6.0, H-8β), 2.92 (1H, ddd, J = 14.8, 4.5, 2.1, H-8α), 4.08 (2H, br s, H-12), 4.98 (1H, br d, J = 11.6, H-6); $[\alpha]^{24}D$ −6.10 (c = 0.86). *Anal.* calcd for $C_{15}H_{21}O_3Br$, C 54.72, H 6.43, Br 24.27; found C 54.53, H 6.46, Br 24.00.

12-ACETOXY-4α-HYDROXYEUDESM-7(11)-ENO-13,6α-LACTONE [**9**].—A mixture of **7** (161 mg,

0.49 mmol) and AgOAc (105 mg, 0.63 mmol) in DMF (9 ml) was stirred at room temperature for 5 h and filtered. The filtrate was poured into saturated aqueous $NaHCO_3$ (20 ml) and extracted with EtOAc (50 ml, 2 × 20 ml). The combined extracts were washed with saturated aqueous NaCl (20 ml), dried (Na_2SO_4), and concentrated to give a pale yellow oil (500 mg), which was chromatographed over Si gel (15 g, 1.5 cm i.d. column). Elution with hexane-EtOAc (2:1) gave spectroscopically pure **9** (111 mg, 74%) as colorless crystals, which were recrystallized from hexane-Et_2O (1:1) to give colorless prisms: mp 62–63°; ir (KBr) 3570, 1750, 1685 cm^{-1}; ^1H nmr (90 MHz) δ 1.08 (3H, s, 10-Me), 1.41 (3H, s, 4-Me), 2.06 (3H, s, -OAc), 2.52 (1H, ddd, J = 14.7, 13.1, 5.9, H-8β), 2.98 (1H, ddd, J = 14.7, 4.5, 2.4, H-8α), 4.78 (2H, br s, H-12), 4.97 (1H, br d, J = 11.8, H-6); $[\alpha]^{24}D$ −6.31 (c = 1.87). *Anal.* calcd for $C_{17}H_{24}O_5$, C 66.21, H 7.84; found C 65.87, H 7.95.

REACTION OF **5** WITH AgOAc: DIRECT FORMATION OF **9** FROM **5**.—A mixture of **5** (625 mg, 1.52 mmol) and AgOAc (813 mg, 4.88 mmol) in DMF (28 ml) was stirred at room temperature for 23 h, poured into saturated aqueous $NaHCO_3$ (20 ml), and filtered through celite. The filtrate was extracted with EtOAc (50 ml, 2 × 20 ml). The extract was washed with saturated aqueous NaCl (20 ml), dried (Na_2SO_4), and concentrated to give a pale yellow oil, which was chromatographed over Si gel (50 g, 3 cm i.d. column) with hexane-EtOAc (2:1).

The first fraction gave 12-bromo-4α-hydroxyeudesm-11(12)-eno-13,6α-lactone [**11**] (40 mg, 8%) as a colorless solid, which was recrystallized from hexane-EtOAc (1:1) to give colorless prisms: mp 165°; ir (KBr) 3670, 1760, 1640 cm^{-1}; ^1H nmr (200 MHz) δ 1.00 (3H, s, 10-Me), 1.33 (3H, s, 4-Me), 1.83 (1H, d, J = 11.8, H-5), 2.61 (1H, m, H-8α), 2.69 (1H, dddd, J = 11.8, 10.4, 3.2, 3.2 Hz, H-7), 4.14 (1H, dd, J = 11.8, 10.4, H-6), 7.42 (1H, d, J = 3.2, H-12). *Anal.* calcd for $C_{15}H_{21}O_3Br$, C 54.72, H 6.43, Br 24.27; found C 55.02, H 6.46, Br 24.66.

The second fraction gave **9** (322 mg, 69%).

4α,12-DIHYDROXYEUDESM-7(11)-ENO-13,6α-LACTONE (ARBUSCLIN D [**1**]).—A mixture of 9 (18 mg, 0.06 mmol), 0.2 M aqueous Na_2CO_3 (0.75 ml) in MeOH was stirred at room temperature for 13 min, poured into saturated NH_4Cl (10 ml), and extracted with EtOAc (4 × 10 ml). The combined extracts were washed with saturated aqueous NaCl, dried ($MgSO_4$), and concentrated to give a colorless oil (12 mg). This crude product was purified by hplc [B, EtOAc-hexane (1:1), 5, Rt 13.6] to give spectroscopically pure **1** (10 mg, 63%) as a colorless solid, which was subsequently crystallized from EtOAc to give colorless needles: mp 174–175°; ir (KBr) 3540, 3425, 1753, 1685 cm^{-1}; ^1H nmr (90 MHz) δ 1.07 (3H, s, 10-Me), 1.41 (3H, s, 4-Me), 1.49 (3H, d, J = 11.3, H-5), 2.48 (1H, ddd, J = 15.0, 13.2 6.6, H-8β), 2.88 (1H, ddd, J = 15.0, 5.3, 2.4, H-8α), 3.23 (1H, br s, -O*H*), 4.36 (2H, br s, H-12), 4.95 (1H, br d, J = 11.3, 6-H); $[\alpha]^{24}D$ −38.4 (c = 1.16). *Anal.* calcd for $C_{15}H_{22}O_4$, C 67.65, H 8.33; found C 67.69, H 8.24.

11β,12-DIBROMO-4β-HYDROXYEUDESMANO-13,6α-LACTONE [**6**].—To a stirred mixture of **4** (38 mg, 0.15 mmol) and NaOAc (19 mg, 0.23 mmol) in CH_2Cl_2 (2 ml) was slowly added Br_2 (8.6 μl, 0.17mmol) at 0°. The mixture was stirred for 10 min at 0° and for 1 h at room temperature poured into saturated aqueous $NaHCO_3$ (5 ml), and extracted with $CHCl_3$ (3 × 15 ml). The extracts were washed with saturated aqueous NaCl (50 ml), dried (Na_2SO_4), and concentrated to give a colorless oil (58 mg), which was chromatographed over Si gel (25 g) and eluted with hexane-EtOAc (9:1) to give spectroscopically pure **6** (48.5 mg, 78%) as a colorless oil: ir ($CHCl_3$) 3600, 1790 cm^{-1}; ^1H nmr (90 MHz) δ 1.19 (3H, s, 10-Me), 1.42 (3H, s, 4-Me), 1.64 (1H, d, J = 11.4, H-5), 2.28 (1H, m, H-7), 3.82 (1H, d, J = 11.0, H-12), 4.16 (1H, d, J = 11.0, H-12), 4.52 (1H, dd, J = 11.4, 9.2, H-6); $[\alpha]^{24}D$ +30.2 (c = 0.52).

12-BROMO-4β-HYDROXYEUDESM-7(11)-ENO-13,6α-LACTONE [**8**].—A mixture of **6** (58 mg, 0.12 mmol), Li_2CO_3 (22 mg, 0.29 mmol), and LiBr (16 mg, 0.18 mmol) in DMF (2.5 ml) was stirred at 75° for 45 min, cooled to room temperature, poured into 1 M aqueous H_2SO_4 (10 ml), and extracted with EtOAc (20 ml, 2 × 25ml). The combined extracts were washed successively with saturated aqueous $NaHCO_3$ (5 ml) and saturated aqueous NaBr (5 ml), dried ($MgSO_4$), and concentrated to give a pale yellow oil (76 mg), which was chromatographed over Si gel (3.5 g, 1.2 cm i.d. column). Elution with hexane-EtOAc (3:1) gave spectroscopically pure **8** (30 mg, 78%) as a white solid, which was recrystallized from hexane-EtOAc (1:1) to give colorless needles: mp 150–151°; ir (KBr) 3570, 1765, 1675 cm^{-1}; ^1H nmr (90 MHz) δ 1.24 (3H, s, 10-Me), 1.44 (3H, s, 4-Me), 2.64 (2H, m, H-8), 4.10 (2H, s, H-12), 5.12 (1H, br d, J = 11.7, H-6).

12-ACETOXY-4β-HYDROXYEUDESM-7(11)-ENO-13,6α-LACTONE [**10**].—A mixture of **8** (15 mg, 0.047 mmol) and AgOAc (10 mg, 0.061 mmol) in DMF (0.5 ml) was stirred at room temperature for 18 h, poured into saturated aqueous NaCl (10 ml), and filtered through celite. The filtrate was extracted with EtOAc (20 ml, 2 × 15 ml). The combined extracts were dried ($MgSO_4$) and concentrated to give a crude oily product, which was purified by hplc [B, hexane-EtOAc (6:4), 7.5]. The second peak (Rt 4.8) gave

spectroscopically pure **10** (3.4 mg, 24%), which was subsequently recrystallized from a mixture of hexane and EtOAc to give colorless plates: mp 168°; ir (KBr) 3505, 1745, 1740, 1690 cm^{-1}; ^1H nmr (90 MHz) δ 1.23 (3H, s, 10-Me), 1.45 (3H, s, 4-Me), 2.05 (3H, s, -OAc), 2.56 (1H, ddd, J = 15.5, 12.3, 5.3, H-8β), 2.92 (1H, ddd, J = 15.5, 5.3, 2.3, H-8α), 4.78 (2H, br s, H-12), 5.09 (1H, br d, J = 11.6, H-6); $[\alpha]^{24}D$ +25.2 (c = 0.27). *Anal.* calcd for $C_{17}H_{24}O_5$, C 66.21, H 7.84; found C 66.45, H 8.00.

THE REACTION OF **6** WITH AgOAc: DIRECT FORMATION OF **10** FROM **6**.—A mixture of **6** (13 mg, 0.032 mmol) and AgOAc (17 mg, 0.102 mmol) in DMF (1 ml) was stirred at room temperature for 24 h, poured into saturated aqueous $NaHCO_3$ (5 ml), and filtered through celite. The filtrate was extracted with EtOAc (20 ml, 2 × 15 ml). The extract was washed with saturated aqueous NaCl (20 ml), dried ($MgSO_4$), and concentrated to give a pale yellow oil, which was purified by hplc [A, hexane-EtOAc (6:4), 3, Rt 4.2 min] to give spectroscopically pure **10** (3.3 mg, 34%) as colorless crystals.

4β,12-DIHYDROXYEUDESM-7(11)-ENO-13,6α-LACTONE [**2**].—A mixture of **10** (17 mg, 0.55 mmol) and 0.2 M aqueous Na_2CO_3 (1.3 ml) in MeOH (2.6 ml) was stirred at room temperature for 10 min, poured into a mixture of saturated aqueous NH_4Cl (5 ml) and NaCl (1 g), and extracted with EtOAc (20 ml, 2 × 15 ml). The combined extracts were washed with saturated aqueous NaCl, dried ($MgSO_4$), and concentrated to give a colorless oil (17 mg), which was purified by hplc [B, hexane-EtOAc (1:1), 7.5]. The major peak (Rt 8) gave **2** (11 mg, 73%) as a colorless solid, which was recrystallized from a mixture of hexane and EtOAc to give colorless prisms: mp 188°; ir (KBr) 3380, 2930, 1735, 1675 cm^{-1}; ^1H nmr (90 MHz) δ 1.23 (3H, s, 10-Me), 1.45 (3H, s, 4-Me), 2.64 (2H, m, H-8), 4.38 (2H, br s, H-12), 5.10 (1H, br d, J = 10.4, H-6); $[\alpha]^{24}D$ +11.3 (c = 0.83); hrms m/z 267.1595 ($C_{15}H_{22}O_4$ requires 267.1595).

12-CHLORO-4α-HYDROXYEUDESM-7(11)-ENO-13,6α-LACTONE [**14**].—A mixture of 2 M aqueous HCl (50 ml) and **7** (246 mg, 0.75 mmol) in DMF (14 ml) was stirred for 30 min, poured into saturated aqueous NaCl, and extracted with EtOAc. The extract was washed with saturated aqueous NaCl, dried ($MgSO_4$), and concentrated to give a crude product as a pale yellow oil, which was chromatographed over Si gel (30 g) and eluted with a mixture of hexane and EtOAc (3:1) to give **14** (213 mg, 100%) as a colorless oil: ir ($CHCl_3$) 3580, 2945, 1765, 1680, 1395, 1330, 1100, 1005, 910 cm^{-1}; ^1H nmr (90 MHz) δ 1.09 (3H, s, 10-Me), 1.41 (3H, s, 4-Me), 2.55 (1H, ddd, J = 13.5, 13.5, 6.0, H-8β), 2.92 (1H, ddd, J = 13.5, 4.95, 2.25, H-8α), 3.21 (1H, br s, O*H*), 4.24 (2H, br s, H-12), 4.99 (1H, br d, J = 12.2, H-6); eims m/z (rel. int.) $[M]^+$ (^{37}Cl) 286 (0.6), $[M]^+$ (^{35}Cl) 284 (2), 190 (100).

THE CELL GROWTH INHIBITORY BIOASSAY 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, Inc., Hialeah, FL), and the cell growth inhibition ratio (%) was calculated (Table 1).

THE PLANT GROWTH REGULATING BIOASSAY.—Each compound (15 mg) dissolved in 0.3 ml of solvent [Me_2CO-Tween 80 (10:1)] was diluted with H_2O to give a test solution. Three kinds of seeds, *Echinochloa frumentacea*, *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).

ACKNOWLEDGMENTS

We thank Professor K. Kabuto of Tohoku University for a loan of a polarimeter and his help in the measurement of optical rotations and to Dr. H. Hagiwara of Tohoku University for the measurement of hrms. We also thank Mr. T. Kondo, Mr. K. Sasaki, Dr. M. Ueno, Mr. T. Sato, and Mrs. H. Ando of the Instrumental Analysis Center for Chemistry, Tohoku University for the ^1H-nmr and eims spectra and for the microanalyses.

LITERATURE CITED

1. M. Ando, T. Wada, and K. Isogai, *J. Org. Chem.*, **56**, 6235 (1991).
2. G.M. Massanet and F.R. Luis, *J. Org. Chem.*, **56**, 3587 (1991).
3. M.A. Irwin and T.A. Geissman, *Phytochemistry*, **12**, 853 (1973).
4. M.A. Irwin and T.A. Geissman, *Phytochemistry*, **8**, 2411 (1969).
5. M. Ando, K. Isogai, H. Azami, N. Hirata, and Y. Yanagi, *J. Nat. Prod.*, **54**, 1017 (1991).
6. K. Yamakawa, K. Nishitani, and K. Azusawa, *Heterocycles*, **8**, 103 (1977).

Received 27 April 1992