Acknowledgment. This research was supported by the Comisión Asesora de Investigación Científica y Técnica (CAICYT Project 904/84).

Registry No. 1, 3068-88-0; 2, 625-38-7; 3, 107-93-7; 5, 96-48-0; 6, 3724-55-8; 7, 623-43-8.

Supplementary Material Available: Reaction rates for acid-promoted transformations under heating are summarized in Figures 1-3 (4 pages). Ordering information is given on any current masthead page.

Studies on the Syntheses of Sesquiterpene Lactones. 10.[1] Improved Syntheses of (+)-Tuberiferin and the Related α-Methylene γ-Lactones and Their Biological Activities

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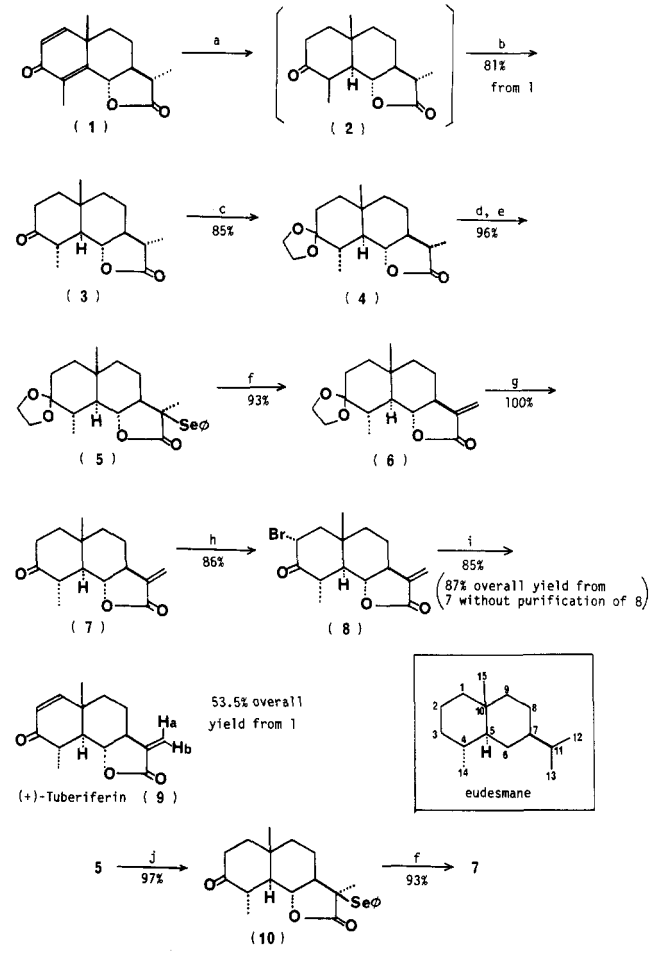
Received January 15, 1987

Sesquiterpene lactones with an α-methylene γ-lactone moiety fused on various skeletons are a rapidly expanding group of natural products, comprising to date more than 900 varieties.[3] Some of them have been shown to have considerable biological activities as allergenic agents, cytotoxic and antitumor agents, regulators of plant growth and antimitotic activity, and antishistosomal agents. Because of their biological activities and because they are available from natural sources often only in small quantities, their efficient syntheses are a synthetic challenge that has received much attention and many reports of such syntheses have appeared in the last decade.

In the course of our program of the study of the structure–activity relationship of α-methylene γ-lactones, we needed efficient syntheses of (+)-tuberiferin (**9**) and related α-methylene γ-lactones in gram quantities. Although total syntheses of (±)- and (+)-tuberiferin (**9**) had already appeared in the literature,[4] the reported methods were inconvenient for our purpose. In the reported total syntheses of (±)- and (+)-**9** the introduction of the α-methylene γ-lactone moiety was left to the final stage, probably because of its expected reactivity and instability.

Since we had noticed that the α-methylene γ-lactone moiety was stable under acidic and mild basic conditions and bromination by phenyltrimethylammonium perbromide (PTAB), we envisioned another approach to **9** in which, as shown in Scheme I, the introduction of the α-methylene γ-lactone moiety was at an early stage. In this note we report efficient syntheses of (+)-tuberiferin (**9**) and the related α-methylene γ-lactones **6**–**8** and their biological activities.

Scheme I[a]



[a] Reagents and conditions: (a) H_2/2% Pd-$SrCO_3$, AcOEt; (b) HCl, EtOH; (c) $HO(CH_2)_2OH$, *p*-TsOH, benzene, reflux; (d) LDA, THF; (e) $(C_6H_5Se)_2$, HMPA, THF; (f) 30% H_2O_2, AcOH, THF; (g) 20% aqueous AcOH–EtOH, reflux; (h) 1.1 equiv of PTAB, THF; (i) Li_2CO_3, LiBr, DMF, 123–131 °C; (j) 50% aqueous AcOH, reflux.

Results and Discussion

Synthesis of (+)-Tuberiferin (9). The starting material **3** was obtained from commercially available *l*-α-santonin (**1**) by modification of the known procedure[5] (Scheme I). Thus, catalytic hydrogenation of **1** over 2% palladium on strontium carbonate in ethyl acetate and epimerization of the resulting **2** possessing a β(axial)-methyl group at C_4 by 2 M hydrochloric acid in ethanol gave **3** in 81% yield. Acetalization of the C_3-carbonyl group of **3** under standard conditions gave acetal **4** in 85% yield. Phenylselenenylation of **4** by Grieco's method[6] gave the corresponding phenyl selenide (**5**) in 96% yield. Treatment of **5** with 30% hydrogen peroxide in THF in the presence of acetic acid gave an α-methylene γ-lactone (**6**) in 93% yield. Deacetalization of **6** was achieved by treatment with 20% aqueous acetic acid in ethanol at 85 °C for 4 h to give **7** in a quantitative yield. **7** was also prepared by a different procedure. Thus, deacetalization of **5** in boiling 50% aqueous acetic acid gave the corresponding keto selenide **10** in 97% yield. Successive treatment of **10** with 30% hydrogen peroxide in THF in the presence of acetic acid gave **7** in 93% yield.

The selective bromination at C_2 of **7** was achieved as follows. Treatment of **7** with 1.05 mol equiv of PTAB[7] in

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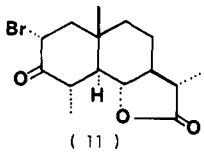
0022-3263/87/1952-4792$01.50/0

Table I. Cell Growth Inhibitory Activity against Murine Lymphocytic Leukemia (P388) in Vitro

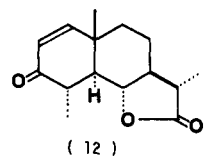
compd	cell growth inhibitory ratio,[a] % 10 μg/mL	1 μg/mL	10^{-1} μg/mL	10^{-2} μg/mL
6	105	98	6	
4	22	5	16	
7	102	40	0	
3	16	7	5	
8	103	106	80	
11	103	69	13	
9	107	102	33	
12	76	14	1	
adriamycin (control)	107	103	104	35

[a] Cell growth inhibitory ratio (%) = $[1 - (T - C_0)/(C - C_0)] \times 100$ where T = cell cound after culture with compound, C = cell count after culture without compound, and C_0 = cell count at the start of culture.

Chart I



(11)



(12)

THF at −6 °C for 1 h gave an α-bromo ketone (8) in 86% yield. The bromine atom at C_2 of 8 was α-equatorial because of the magnitude of the coupling constant of the C_2 proton [δ 4.83 (1 H, ddd, J = 12.8, 6.5, 1.2 Hz)].[8]

Dehydrobromination of 8 with lithium carbonate and lithium bromide in DMF at 123–131 °C for 100 min gave an α,β-unsaturated ketone (9) in 85% yield. 9 was also obtained in 87% overall yield from 7 by using the crude bromination product without any further purification. 9 was identical with (+)-tuberiferin[9] by direct comparison of their ^{1}H NMR spectra (60 MHz, $CDCl_3$).[10] The IR spectrum, melting point, and $[\alpha]_D$ value of 9 were also in good accordance with those of (+)-tuberiferin reported in the literature.[4,9]

Biological Activities. 1. Cell Growth Inhibitory Activity toward the P388 Lymphocytic Leukemia Test System. The α-methylene γ-lactones **6–9** showed significant cell growth inhibitory activity against murine lymphocytic leukemia (P388) in vitro. The results are compared with those of the corresponding α-methyl γ-lactones in Table I. As expected, the α-methylene γ-lactones 6–9 showed stronger activity than the corresponding saturated α-methyl γ-lactones **4**, **3**, **11**, and **12** (Chart I). It is interesting that the α-bromo ketone (11) possessing a saturated α-methyl γ-lactone moiety showed significant cell growth inhibitory activity against P388 lymphocytic leukemia in vitro.

2. Plant Growth Regulating Activity. The plant growth regulating activity of α-methylene γ-lactones **6–9** was studied employing three kinds of seeds, *Echinochloa frumentacea* (Japanese millet, Japanese name shokuyō hie), *Brassica juncea* (brown mustard, Japanese name seiyō karashina), and *Cucumis sativus* (cucumber, Japanese name kyūri). The germination of seeds and growth of seedlings were observed. Compounds 7 and 9 showed significant plant growth inhibitory activity at 1000 ppm. The results are summarized in Table II.

Table II. Plant Growth Inhibitory Activities of Compounds 6–9[a]

compd	plant	part	activities 1000 ppm	100 ppm
6	*E. frumentacea*	stem	+	−
		root	++	−
	B. juncea	stem	− to +	− to +
		root	+	−
	C. sativus	stem	−	−
		root	+	−
7	*E. frumentacea*	stem	++	−
		root	+++	− to +
	B. juncea	stem	+ to ++	−
		root	++ to +++	+
	C. sativus	stem	− to +	−
		root	+++	− to +
8	*E. frumentacea*	stem	−	−
		root	++	−
	B. juncea	stem	−	−
		root	+ to ++	−
	C. sativus	stem	−	−
		root	+	−
9	*E. frumentacea*	stem	+++	− to +
		root	+++	−
	B. juncea	stem	++ to +++	−
		root	+++	−
	C. sativus	stem	++ to +++	−
		root	+++	+

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

3. Control of Crop Diseases. The preventive and curative activities of compounds 4 and 6–9 in controlling crop diseases were examined by pot test. The α-methylene γ-lactones 7–9 showed significant preventive activities in controlling dawny mildew of grape and late blight of tomato. The results are summarized in Table III.

Experimental Section

All melting points were uncorrected. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$. Mass spectra were recorded at 25 eV.

(11*S*)-3-Oxoeudesmano-13,6α-lactone (3). A mixture of *l*-α-santonin (1; 23.10 g, 93.79 mmol), ethyl acetate (750 mL), and 2% palladium on strontium carbonate (10.3 g) was shaken under 1 atm hydrogen at room temperature. The hydrogen uptake ceased at 5.1 L (227.68 mmol) in 41 min. The catalyst was removed, and the filtrate was concentrated to 100 mL to give the first crop of (11*S*)-3-oxo-4α*H*-eudesmano-13,6α-lactone (2, 10.95 g). The filtrate was further concentrated to give the second crop of 2 (5.28 g), which was indistinguishable from the first crop in the comparison of their NMR and IR spectra. The combined yield of 2 was 69.1%. The mother liquid was concentrated to give an oily crude material (10.97 g), which was dissolved in a mixture of ethanol (60 mL) and 2 M HCl (6 mL) and allowed to stand at room temperature for 15 h. The mixture was poured into a saturated aqueous solution of NaCl (150 mL) and extracted with ethyl acetate (4 × 40 mL). The combined extracts were washed with a saturated aqueous solution of NaCl, dried (Na_2SO_4), and concentrated to give a crude crystalline material (3.56 g), which was recrystallized from ethanol to give 3 (2.71 g, 11.5%) as needles.

The combined 2 (16.23 g) obtained above was allowed to stand at room temperature in a mixture of ethanol (60 mL) and 2 M HCl (9 mL) for 15 h and worked up in the above mentioned manner to give 3 in a quantitative yield. Thus, the combined yield of 3 from 1 was 81%.

The analytical sample of 2 was obtained by recrystallization from ethyl acetate as plates: mp 141.5–144.5 °C (lit.[5] mp 145.5–147 °C); IR (KBr) 1765, 1708 cm^{-1}; ^{1}H NMR (60 MHz) δ 1.20 (3 H,

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Table III. Preventive or Curative Activities of Compounds 4 and 6–9 in Controlling Crop Diseases

		evaluation of disease control[a]						
compd	concn, ppm	blast of rice[b]	sheath blight of rice[c]	powdery mildew of wheat[d]	damping off of cucumber[e]	downy mildew of grape[f]	late blight of tomato[g]	scap of apple[h]
4	500	1	3	0	0	0[i]	0	0
6	500	3	0	0	0	0[i]	0	0
7	500	2	0	0	0	0[i]	4	0
	200					4	0	
	50					1	0	
8	500	2	0	1	3	0[i]	4	0
	200					4	4	
	50					0	0	
9	500	1	2	0	0	0[i]	4	1
	200					4	2	
	50					1	0	

[a] This assessment was made by disease severity in rice, wheat, grape, tomato, and apple or numbering infecting seedlings of cucumber. The indices are expressed on a scale of five: 5, 100%; 4, 99–90%, 3, 89–70%, 2, 69–50%, 1, 49–30%, 0, 29–0%. [b] Caused by *Pyricularia oryzae*. [c] Caused by *Rhizoctonia solani*. [d] Caused by *Erysiphe graminis*. [e] Caused by *Pythium aphanidermatum*. [f] Caused by *Plasmopara viticora*. [g] Caused by *Phytophthora infestano*. [h] Caused by *Ventria inaequalis*. [i] Curative activity. The preventive activity was not determined at this concentration.

s, C_{10}-CH_3), 1.23 (3 H, d, J = 6.5 Hz), 1.24 (3 H, d, J = 7.5 Hz), 4.00 (1 H, dd, J = 10.0, 10.0 Hz, C_6-H). Anal. Calcd for $C_{15}H_{22}O_3$: C, 71.97; H, 8.86. Found: C, 71.99; H, 8.61.

The analytical sample of **3** was obtained by recrystallization from a mixture of chloroform and ether (1:1) as needles: mp 153–155 °C (lit.[11] mp 154–155 °C); IR (KBr) 1775, 1698 cm^{-1}; ^1H NMR (60 MHz) δ 1.19 (3 H, s, C_{10}-CH_3), 1.22 (3 H, d, J = 7.2 Hz), 1.25 (3 H, d, J = 7.0 Hz), 3.91 (1 H, dd, J = 11.0, 9.0 Hz, C_6H). Anal. Calcd for $C_{15}H_{22}O_3$: C, 71.97; H, 8.86. Found: C, 71.79; H, 9.06.

(11*S*)-3,3-(Ethylenedioxy)eudesmano-13,6α-lactone (4). A mixture of **3** (1.00 g, 3.99 mmol), ethylene glycol (5 mL, 89.4 mmol), and *p*-toluenesulfonic acid (40.2 mg, 0.21 mmol) in dry benzene was refluxed in a flask equipped with a Dean–Stark column packed with molecular sieves for 24 h. The mixture was cooled, diluted with a saturated aqueous solution of NaCl (100 mL), and shaken, and the benzene layer was drawn off. The aqueous layer was further extracted with benzene (4 × 10 mL). The combined extracts were washed with a saturated aqueous solution of NaCl (3 × 10 mL), dried, and concentrated to give a crystalline crude product, which was recrystallized from a mixture of benzene, ether, and hexane (1:1:1) to give **4** (998 mg, 85%) as colorless prisms: mp 167–168 °C; IR (KBr) 1770 cm^{-1}; ^1H NMR (60 MHz) δ 0.99 (3 H, s, C_{10}-CH_3), 1.04 (3 H, d, J = 6.2 Hz, C_4-CH_3), 1.18 (3 H, d, J = 6.2 Hz, C_{11}-CH_3), 3.81 (1 H, dd, J = 10.5, 10.5 Hz, C_6-H), 3.93 (4 H, br s, OCH_2CH_2O); ^{13}C NMR δ 12.0 (q, C_{15}), 12.5 (q, C_{12}), 18.1 (q, C_{14}), 23.1 (t, C_8), 31.1 (t, C_2), 36.6 (s, C_{10}), 39.0 (t, C_9), 39.4 (d, C_4), 40.6 (d, C_{11}), 40.6 (t, C_1), 50.9 (d, C_5 or C_7), 53.5 (d, C_5 or C_7), 65.1 (t, OCH_2CH_2O), 83.2 (d, C_6), 110.5 (s, C_3), 179.3 (s, C_{13}); MS, m/e (relative intensity) 294 (M^+, 6.9), 99 (100); $[\alpha]^{20}_D$ +24.3° (c 1.14, $CHCl_3$). Anal. Calcd for $C_{17}H_{26}O_4$: C, 69.36; H, 8.90. Found: C, 69.56; H, 9.04.

11β-(Phenylseleno)-3,3-(ethylenedioxy)eudesmano-13,6α-lactone (5). To a THF solution of lithium diisopropylamide [prepared from diisopropylamine (0.28 mL, 2.00 mmol), 1.50 M butyllithium in hexane (1.48 mL, 2.22 mmol), and THF (5.0 mL) at −78 °C] was added dropwise over a period of 1 h 296 mg (1.00 mmol) of **4** in THF (7.5 mL). After the solution was stirred at −78 °C for 75 min, diphenyl diselenide (625 mg, 2.00 mmol) in THF (50 mL) containing HMPA (348 mg, 2.00 mmol) was added dropwise at −78 °C over a period of 50 min. The reaction mixture was stirred at −78 °C for 30 min and then warmed to −40 °C, where stirring was continued for an additional 45 min. The reaction was quenched by the addition of 0.1 M aqueous solution of HCl (25 mL). The mixture was extracted with chloroform (3 × 30 mL). The combined extracts were washed with a saturated aqueous solution of NaCl (2 × 30 mL), dried, and concentrated to give a crude product, which was subsequently chromatographed over silica gel (Merck, 70–230 mesh, 50 g) and eluted with chloroform to give diphenyl diselenide. The fractions eluted with ethyl acetate gave spectroscopically pure **5** (432 mg, 96%) as pale yellow prisms. The analytical sample was obtained by recrystallization from a mixture of benzene and ether (1:1) as pale yellow prisms: mp 180.5–181.5 °C; IR (KBr) 1770 cm^{-1}; ^1H NMR (90 MHz) δ 1.01 (3 H, s, C_{10}-CH_3), 1.04 (3 H, d, J = 6.7 Hz, C_4-CH_3), 1.50 (3 H, s, C_{11}-CH_3), 2.01 (1 H, dq, J = 11.3, 6.7 Hz, C_4-H), 3.93 (4 H, br s, OCH_2CH_2O), 4.24 (1 H, dd, J = 9.8, 9.8 Hz, C_6-H), 7.18–7.38 (3 H), 7.52–7.65 (2 H); ^{13}C NMR δ 12.0 (q, C_{15}), 18.1 (q, C_{14}), 21.0 (t, C_8), 22.3 (q, C_{12}), 31.1 (t, C_2), 36.5 (s, C_{10}), 38.8 (t, C_9), 39.4 (d, C_4), 40.5 (t, C_1), 48.7 (s, C_{11}), 51.0 (d, C_5), 57.8 (d, C_7), 65.1 (t, OCH_2CH_2O), 81.1 (d, C_6), 110.4 (s, C_3), 124.4 (s, C_6H_5), 128.9 (d, C_6H_5), 129.5 (d, C_6H_5), 138.1 (d, C_6H_5), 176.6 (s, C_{13}); MS, m/e (relative intensity) 452 [M^+ (^{82}Se), 1], 450 [M^+ (^{80}Se), 4], 448 [M^+ (^{78}Se), 3], 447 [M^+ (^{77}Se), 1], 446 [M^+ (^{76}Se), 1], 292 (77), 99 (100). $[\alpha]^{20}_D$ +33.1° (c 2.00, $CHCl_3$). Anal. Calcd for $C_{23}H_{30}O_4Se$: C, 61.46; H, 6.73. Found: C, 61.51; H, 6.90.

3,3-(Ethylenedioxy)eudesm-11(12)-eno-13,6α-lactone (6). A solution of **5** (592 mg, 1.32 mmol) in THF (25 mL) containing acetic acid (0.200 mL, 3.74 mmol) was treated at 0 °C with 30% H_2O_2 (0.90 mL, 8.83 mmol) for 1.5 h. The reaction mixture was poured into a cold saturated aqueous solution of $NaHCO_3$ (40 mL) and extracted with ethyl acetate (5 × 25 mL). The combined extracts were washed with a saturated aqueous solution of NaCl (3 × 50 mL), dried (Na_2SO_4), and concentrated to give 382 mg of crude crystalline product, which was recrystallized from ethyl acetate to give spectroscopically pure **6** as colorless crystals (359 mg, 93%). The analytical sample of **6** was obtained by the recrystallization from a mixture of benzene, ether, and hexane (1:1:1) as prisms: mp 161–163 °C; IR (KBr) 1775 cm^{-1}; ^1H NMR (90 MHz) δ 0.98 (3 H, s, C_{10}-CH_3), 1.08 (3 H, d, J = 6.0 Hz, C_4-CH_3), 1.73 (1 H, dd, J = 10.5, 10.5 Hz, C_5-H), 2.02 (1 H, dq, J = 10.5, 6.0 Hz, C_4-H), 2.49 (1 H, ddddd, J = 10.5, 10.5, 3.0, 3.0, 3.0 Hz, C_7-H), 3.78 (1 H, dd, J = 10.5, 10.5 Hz, C_6-H), 3.96 (4 H, br s, OCH_2CH_2O), 5.34 (1 H, d, J = 3.0 Hz, C_{12}-H_a), 6.01 (1 H, d, J = 3.0 Hz, C_{12}-H_b); ^{13}C NMR δ 12.01 (q, C_{15}), 18.1 (q, C_{14}), 21.5 (t, C_8), 31.0 (t, C_2), 36.6 (s, C_{10}), 39.0 (t, C_9), 39.2 (d, C_4), 40.2 (t, C_1), 50.8 (d, C_5 or C_7), 51.6 (d, C_5 or C_7), 65.1 (t, OCH_2CH_2O), 83.4 (d, C_6), 110.4 (s, C_3), 116.4 (t, C_{12}), 139.3 (s, C_{11}), 170.6 (s, C_{13}); MS, m/e (relative intensity) 292 (M^+, 100), 99 (45); $[\alpha]^{20}_D$ +29.6° (c 2.93, $CHCl_3$). Anal. Calcd for $C_{17}H_{24}O_4$: C, 69.83; H, 8.27. Found: C, 69.79; H, 8.41.

(11*S*)-3-Oxoeudesm-11(12)-eno-13,6α-lactone (7). A mixture of **6** (52 mg, 0.18 mmol), ethanol (4.5 mL), and a 20% aqueous solution of acetic acid (4.5 mL) was refluxed (bath temperature 85 °C) for 4 h. The mixture was cooled, poured into a saturated aqueous solution of NaCl (20 mL), and extracted with ethyl acetate (4 × 10 mL). The combined extracts were washed successively with a saturated aqueous solution of $NaHCO_3$ (2 × 10 mL) and a saturated aqueous solution of NaCl (3 × 10 mL), dried (Na_2SO_4), and concentrated to give spectroscopically pure **7** (44 mg, 100%) as a colorless crystalline material (mp 153–156 °C), which was recrystallized from ethyl acetate to give colorless needles: mp 171–173 °C; IR (KBr) 1762, 1704 cm^{-1}; ^1H NMR (90 MHz) δ 1.18 (3 H, s, C_{10}-CH_3), 1.28 (3 H, d, J = 6.8 Hz, C_4-CH_3), 1.69 (1 H, dd, J = 10.5, 10.5 Hz, C_5-H), 2.53 (1 H, dq, J = 10.5, 6.8 Hz, C_4-H),

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Table IV. Pot Test Procedures

disease (pathogen)	host	test compounds application method	dosage,[a] ppm	inoculation method	incubation method for disease development	assessment method of disease severity
blast (*P. oryzae*)	rice	preventive foliar spray a few hours preinoculn	500	foliar spray of spore suspension	4 days, high humidity, darkness, 28 °C	evaluation by infection indices (0–5) depending on number of brown spots
sheath blight (*R. solani*)	rice	preventive foliar spray a few hours preinoculn	500	infestation of water with mycelium grown in the chaff's medium	4 days, high humidity, darkness, 28 °C	evaluation by infection indices (0–5) depending on size of necrotic lesions
powdery mildew (*E. graminis*)	wheat	preventive foliar spray a few hours preinoculn	500	dusting of spores	10 days, fluorescent lamps, 22 °C	evaluation by infection indices (0–5) depending on rate of leaf coverage of powdery mildew
damping off (*P. aphanidermatum*)	cucumber	solid drench immed postsowing	500 20 mL/pot	infestation of soil with mycelium grown in the bran's medium	14 days, greenhouse, ~25 °C	evaluation by infection indices (0–5) depending on number of infected seedlings
dawny mildew (*P. viticora*)	grape	preventive foliar spray 1 day preinoculn	200, 50	foliar spray of spore suspension	3 days, high humidity, darkness; 7 days, under natural condition, greenhouse	evaluation by infection indices (0–5) depending on rate of necrosis
		curative foliar spray 16–18 h postinoculn	500	foliar spray of spore suspension	3 days, high humidity, darkness; 7 days, under natural condition, greenhouse	evaluation by infection indices (0–5) depending on rate of necrosis
late blight (*P. infestano*)	tomato	preventive foliar spray a few hours preinoculn	500	foliar spray of spore suspension	7 days, high humidity, darkness, 20 °C	evaluation by infection indices (0–5) depending on rate of necrosis
scab (*V. inaequalis*)	apple	preventive foliar spray a few hours preinoculn	500	foliar spray of spore suspension	5 days, high humidity, darkness, 15 °C; 10 days, fluorescent lamps	evaluation by infection indices (0–5) depending on rate of scab lesions

[a] Active ingredient.

3.87 (1 H, dd, J = 10.5, 10.5 Hz, C_6-H), 5.40 (1 H, d, J = 3.0 Hz, C_{12}-H_a), 6.05 (1 H, d, J = 3.3 Hz, C_{12}-H_b); ^{13}C NMR δ 13.8 (q, C_{15}), 18.3 (q, C_{14}), 21.4 (t, C_8), 36.6 (s, C_{10}), 37.2 (t, C_9), 39.8 (t, C_1 or C_2), 40.6 (t, C_1 or C_2), 44.6 (d, C_4), 50.1 (d, C_5 or C_7), 54.0 (d, C_5 or C_7), 83.2 (d, C_6), 117.1 (t, C_{12}), 138.6 (s, C_{11}), 170.1 (s, C_{13}), 210.9 (s, C_3); MS m/e (relative intensity) 248 (M^+, 43), 137 (36), 124 (28), 110 (64), 82 (22), 58 (20), 43 (48), 28 (73), 18 (100); $[\alpha]^{20}_D$ +39.6° (c 2.26, $CHCl_3$). Anal. Calcd for $C_{15}H_{20}O_3$: C, 72.55; H, 8.12. Found: C, 72.47; H, 8.14.

(11*S*)-11β-(Phenylseleno)-3-oxoeudesmano-13,6α-lactone (10). A mixture of **5** (3.375 g, 7.51 mmol) and a 50% aqueous solution of acetic acid (60 mL) was refluxed (bath temperature 120 °C) for 20 min. The mixture was cooled, poured into a saturated aqueous solution of NaCl (150 mL), and extracted with ethyl acetate (4 × 150 mL). The combined extracts were washed successively with a saturated aqueous solution of $NaHCO_3$ (3 × 300 mL) and a saturated aqueous solution of NaCl (2 × 300 mL), dried (Na_2SO_4), and concentrated to give a crystalline material, which was recrystallized from ethyl acetate to give pale yellow needles: 2.955 g, 97%; mp 170–171 °C; IR (KBr) 2935, 1765, 1710 cm^{-1}; ^1H NMR (90 MHz) δ 1.19 (3 H, s, C_{10}-CH_3), 1.24 (3 H, d, J = 6.9 Hz, C_4-CH_3), 1.51 (3 H, s, C_{11}-CH_3), 1.65 (1 H, t, J = 10.5 Hz, C_5-H), 2.50 (1 H, dq, J = 10.5, 6.9 Hz, C_4-H), 4.32 (1 H, dd, J = 10.5, 9.8 Hz, C_6-H), 7.20–7.65 (5 H, m, C_6H_5); ^{13}C NMR δ 13.8 (q, C_{15}), 18.2 (q, C_{14}), 20.8 (t, C_8), 22.2 (q, C_{12}), 36.4 (s, C_{10}), 37.1 (t, C_9), 39.9 (t, C_1 or C_2), 40.5 (t, C_1 or C_2), 44.8 (d, C_4), 48.4 (s, C_{11}), 53.5 (d, C_5), 57.0 (d, C_7), 80.9 (d, C_8), 124.2 (s, C_6H_5), 128.9 (d, C_6H_5), 129.6 (d, C_6H_5), 138.0 (d, C_6H_5), 176.1 (s, C_{13}), 210.9 (s, C_3); $[\alpha]^{20}_D$ +43.7° (c 1.25, $CHCl_3$); MS m/e (relative intensity) 408 [M^+ (^{82}Se), 20], 406 [M^+ (^{80}Se), 83], 404 [M^+ (^{78}Se), 53], 403 [M^+ (^{77}Se), 18], 402 [M^+ (^{76}Se), 16], 400 [M^+ (^{74}Se), 3], 249 (100), 248 (68), 231 (30), 175 (27), 158 (27), 111 (53). Anal. Calcd for $C_{21}H_{26}O_3Se$: C, 62.22; H, 6.46. Found: C, 62.50; H, 6.62.

Conversion of 10 to 7. A solution of **10** (2.924 g, 7.21 mmol) in THF (45 mL) containing acetic acid (1 mL, 17.5 mmol) was treated at 0 °C with 30% H_2O_2 (5.2 mL, 51 mmol) for 1.5 h. The reaction mixture was poured into a cold saturated aqueous solution of $NaHCO_3$ (150 mL) and extracted with ethyl acetate (2 × 150 mL, 2 × 100 mL). The combined extracts were washed with a 1 M solution of KI (100 mL), a 10% aqueous solution of $Na_2S_2O_3$ (1 × 100 mL), and a saturated aqueous solution of NaCl (3 × 150 mL), dried (Na_2SO_4), and concentrated to give a crystalline material, which was recrystallized from ethyl acetate to give **7** as colorless prisms (1.672 g, 93%).

2α-Bromo-3-oxoeudesm-11(12)-eno-13,6α-lactone (8). The solution of phenyltrimethylammonium perbromide (PTAB; 2.167 g, 5.76 mmol) in THF (30 mL) was added dropwise to the stirred solution of **7** (1.364 g, 5.49 mmol) in THF (50 mL) at −6 °C over a period of 5 min. The mixture was stirred at −6 °C for 1 h and then poured into a mixture of a saturated solution of $NaHCO_3$ (100 mL) and 0.2 M aqueous solution of $Na_2S_2O_3$ (100 mL). The mixture was extracted with ethyl acetate (5 × 90 mL). The combined extracts were washed with a saturated aqueous solution of NaCl (3 × 200 mL), dried, and concentrated to give a crude product as pale yellow crystals, which was recrystallized from ethyl acetate to give colorless prisms: 1.542 g, 86%; mp 147–8 °C dec; IR (KBr) 1760, 1720, 1670, 1630 cm^{-1}; ^1H NMR (90 MHz) δ 1.28 (3 H, s, C_{10}-CH_3), 1.38 (3 H, d, J = 6.5 Hz, C_4-CH_3), 1.77 (1 H, dd, J = 11.3, 10.5 Hz, C_5-H), 2.09 (1 H, dd, J = 12.8, 12.8 Hz, C_1-H_α), 2.43 (1 H, dd, J = 12.8, 6.5 Hz, C_1-H_β), 2.73 (1 H, dq, J = 11.3, 6.6 Hz, C_4-H), 3.87 (1 H, dd, J = 10.5, 10.5 Hz, C_6-H), 4.83 (1 H, ddd, J = 12.8, 6.5, 1.2 Hz, C_2-H), 5.42 (1 H, d, J = 3.0 Hz, C_{12}-H_a), 6.08 (1 H, d, J = 3.3 Hz, C_{12}-H_b); ^{13}C NMR δ 14.1 (q, C_{15}), 18.8 (q, C_{14}), 21.1 (t, C_8), 39.2 (s, C_{10}), 39.2 (t, C_9), 44.8 (d, C_4),

50.1 (d, C_5 or C_7), 52.8 (d, C_5 or C_7), 53.2 (t, C_1), 54.4 (d, C_2), 82.3 (d, C_6), 117.6 (t, C_{12}), 138.2 (s, C_{11}), 169.9 (s, C_{12}), 201.2 (s, C_3); MS (13.5 eV), *m/e* (relative intensity) 328 [M^+ (^{81}Br), 12] 327 [M^+ (^{81}Br) – 1, 43], 326 [M^+ (^{79}Br), 16], 325 [M^+ (^{79}Br) – 1, 33], 248 (24), 246 (54), 231 (57), 229 (100), 220 (39), 219 (36); $[\alpha]^{20}_D$ +26.4° (*c* 1.46, $CHCl_3$). Anal. Calcd for $C_{15}H_{19}O_3Br$: C, 55.06; H, 5.81. Found: C, 54.82; H, 5.95.

Tuberiferin (9). A mixture of **8** (450 mg, 1.38 mmol), Li_2CO_3 (270 mg, 3.65 mmol), and LiBr (205 mg, 2.36 mmol) in anhydrous DMF (15 mL) was stirred at 123–131 °C for 1.5 h, cooled to room temperature, poured into a saturated aqueous solution of NH_4Cl (15 mL), and extracted with ethyl acetate (5 × 20 mL). The combined extracts were washed with a saturated aqueous solution of NaCl (3 × 50 mL), dried (Na_2SO_4), and concentrated to give a crystalline material, which was recrystallized from ethyl acetate to give spectroscopically pure **9** (289 mg, 85%). The analytical sample of **9** was obtained by further recrystallization from ethyl acetate as prisms: mp 179–181 °C;[12] IR (KBr)[12] 3085, 3025, 1763, 1665, 1625, 1410, 1250, 1235, 1142, 997, 965, 945, 830 cm^{-1}; ^1H NMR (90 MHz) δ 1.17 (3 H, s, C_{10}-CH_3), 1.39 (3 H, d, *J* = 6.8 Hz, C_4-CH_3), 2.08 (1 H, dd, *J* = 10.5, 12.0 Hz, C_5-H), 2.58 (1 H, dq, *J* = 12.0, 6.8 Hz, C_4-H), 3.96 (1 H, dd, *J* = 10.5, 10.5 Hz, C_6-H), 5.41 (1 H, d, *J* = 3.0 Hz, C_{12}-H_a), 5.85 (1 H, d, *J* = 10.0 Hz, C_2-H), 6.05 (1 H, d, *J* = 3.3 Hz, C_{12}-H_b), 6.68 (1 H, d, *J* = 10.0 Hz, C_1-H); ^{13}C NMR δ 14.6 (q, C_{15}), 19.2 (q, C_{14}), 21.1 (t, C_8), 37.1 (t, C_9), 38.4 (s, C_{10}), 41.9 (d, C_4), 50.1 (d, C_5 or C_7), 52.2 (d, C_5 or C_7), 81.9 (d, C_6), 117.2 (t, C_{12}), 126.4 (d, C_2), 138.3 (s, C_{11}), 158.0 (d, C_1), 170.0 (s, C_{13}), 200.2 (s, C_3); MS (13.5 eV), *m/e* (relative intensity) 246 (M^+, 100), 218 (74), 192 (27), 190 (20); $[\alpha]^{20}_D$ +12.2° (*c* 2.12, $CHCl_3$). Anal. Calcd for $C_{15}H_{18}O_3$: C, 73.15; H, 7.37. Found: C, 72.98; H, 7.33.

When the crude sample of **9** was chromatographed over silica gel and eluted successively with chloroform and ethyl acetate, the eluent with ethyl acetate gave a crystalline material, mp 155–157 °C.[13,14] Although the ^1H NMR spectrum in $CDCl_3$ and IR spectrum in chloroform were identical with those of prisms, mp 179–181 °C, above mentioned, the IR spectrum recorded as KBr disk was apparently different: IR (KBr)[13] 3100, 3040, 2950, 1765, 1680, 1622, 1412, 1255, 1240, 1150, 1140, 1000, 990, 950, 838 cm^{-1}; IR ($CHCl_3$) 3025, 2945, 1765, 1670, 1410, 1245, 1137, 998, 967, 823 cm^{-1}.

Synthesis of Tuberiferin (9) from 7 without Purification of 8. The solution of PTAB (255 mg, 0.677 mmol) in THF (3.0 mL) was added to the stirred solution of **7** (150 mg, 0.605 mmol) in THF (6 mL) at –2 °C over a period of 5 min. The mixture was stirred for 1 h at this temperature and worked up as usual to give 216 mg of crude **8**.

The mixture of this crude **8** (216 mg), Li_2CO_3 (131 mg, 1.77 mmol), and LiBr (98 mg, 1.13 mmol) in anhydrous DMF (6.5 mL) was stirred at 123–131 °C for 1.5 h and worked up as usual to give a crystalline material (183 mg), which was purified by column chromatography [Merck, silica gel, 70–250 mesh, 15 g, AcOEt–$CHCl_3$ (1:9)] to give spectroscopically pure **9** (130 mg, 87% overall yield from **7**).

Cell Growth Inhibitory Activity of Compounds to Murine Lymphocytic Cell (P388) in Vitro. Murine lymphocytic leukemia cells (P388) were incubated with compounds at 37 °C in humidified atmosphere of 5% CO_2 for 48 h. After incubation, the cell number was counted with a Coulter counter (Model ZBI, Coulter Electronics, Inc., Haialeah, FL), and the cell growth inhibition ratio (%) was calculated according to

$$\text{cell growth inhibn ratio (\%)} = \left(1 - \frac{T - C_0}{C - C_0}\right) \times 100$$

where T = cell count after culture with compound, C = cell count after culture without compound, and C_0 = cell count at the start of culture.

Plant Growth Regulation Activity of Compounds. The compounds (15 mg) dissolved in 0.3 mL of solvent (acetone–Tween 80, 10:1) were diluted with water to give test solutions. Three kinds of seeds, *E. frumentacea*, *B. juncea*, and *C. sativus*, were sown in a Petri dish (10 cm × 15 cm) containing 10 mL of the test solution and incubated under light (4000 lx) at 27 °C for 10 days. The germination of seeds and the growth of seedlings were observed and examined. Effects of compounds on plants were expressed as four rating scales (+++, serious or complete inhibition; ++, obvious effect; +, slight detectable effect; –, no effect).

Preventive or Curative Activity of Compounds in Controlling Crop Diseases. The diseases and the test methods are shown in Table IV. Test samples, which were formulated as emulsifiable in water, were applied by spraying to the plants or drenching to soil before or after inoculation. The plants were inoculated with spores or hypha of fungal pathogens. After incubation, disease severity of test plants was observed under desirable conditions for 4–15 days. The activity was expressed as a score from 0 through 5 (0, 0–29%; 1, 30–49%; 2, 50–69%; 3, 70–89%; 4, 90–99%; 5, 100%; controlled vs. untreated).

Acknowledgment. We express our thanks to Professor S. Yamaguchi for a loan of a polarimeter and for his help in the measurement of optical rotation.

Registry No. **1**, 481-06-1; **2**, 14804-46-7; **3**, 13902-54-0; **4**, 66767-50-8; **5**, 66726-14-5; **6**, 110193-06-1; **7**, 66726-13-4; **8**, 92214-65-8; **9**, 18375-00-3; **10**, 99297-23-1; **11**, 62326-48-1; **12**, 39813-41-7.

(12) Melting point and IR (KBr) data recorded here are in good accordance with those reported by K. Yamakawa et al.[4a]

(13) Melting point and IR (KBr) data recorded here are in good accordance with those reported by J. B. Barrera et al.[9]

(14) A possible explanation is that (+)-tuberiferin has two crystal forms whose melting points and IR spectra in KBr disk are different from each other.

Activation of Zinc by Trimethylchlorosilane: An Improved Procedure for the Preparation of β-Hydroxy Esters from Ethyl Bromoacetate and Aldehydes or Ketones (Reformatsky Reaction)

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Received March 11, 1987

The Reformatsky reaction is the Reformatsky generally applicable procedure for converting aldehydes and ketones to β-hydroxy esters (Scheme I).[1-5]

Scheme I

$$\text{RCOR}' + \text{BrCH}_2\text{COOC}_2\text{H}_5 \xrightarrow[(2)\text{H}_2\text{O}]{(1)\ \text{Zn}} \text{RC(OH)(R}')\text{CH}_2\text{COOC}_2\text{H}_5$$

Normally the reaction is carried out at reflux temperatures by adding carbonyl substrate and bromo ester simultaneously to a suspension of zinc in an appropriate solvent, generally benzene or an ether–benzene mixture. Numerous variations have been proposed in order to improve both the yield and the purity of the hydroxy esters formed.[6-11]

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