Guaianolides as Immunomodulators. Synthesis and Biological Activities of Dehydrocostus Lactone, Mokko Lactone, Eremanthin, and Their Derivatives

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The naturally occurring guaianolides, namely mokko lactone (**1**), dehydrocostus lactone (**2**), eremanthin (**3**), and related guaianolides, **16**, **17**, **21**, **22**, **28**−**31**, **33**, **36**, **37**, and **39**, have been synthesized starting from *l*-α-santonin in an effort to examine their structure−activity relationship as inhibitors of the killing function of cytotoxic T lymphocytes (CTL) and the induction of intercellular adhesion molecule-1 (ICAM-1). It was observed during the present study that the guaianolides possessing an α-methylene γ-lactone moiety, i.e., **2**, **3**, **30**, **33**, **37**, and **39**, exhibited significant inhibitory activity toward the killing function of CTL and the induction of ICAM-1.

The guaianolides represent one of the largest groups of sesquiterpene lactones covering over 500 known naturally occurring compounds.[1] Some of these compounds have been reported to possess high antitumor,[2−7] antishistosomal,[2,8] anthelmintic,[9] contraceptive,[10] root-growth stimulatory,[2,11] root-growth and germination inhibitory activities,[12] and antiulcer activity.[13,14] This diverse bioactivity of guaianolides makes them attractive synthetic targets since the availability of these compounds from natural sources is very limited. It is important to make these natural products available in substantial quantities to explore in detail their bioactivities. Therefore, it is of interest to synthesize these compounds from easily available starting materials.

Among the guaianolides, Mokko lactone (**1**) and dehydrocostus lactone (**2**) were initially isolated from mokko (*Sausurea lappa* Clarke),[15,16] a plant that is used for medicinal purposes. Subsequent work from various laboratories[17,18] led the structures of **1** and **2**. The related compound eremanthin (**3**) was isolated from the heartwood oil of *Eremanthus elaeagnus* and *Vanillosposis erythroppa*.[8,19] It is interesting to note that **3** showed strong prophylactic action against the human parasite *Schistosoma mansoni*.

As a part of our ongoing research program, we screened ∼100 herbal medicines for inhibitors of the killing function of cytotoxic T lymphocyte (CTL) and found that only the extract of *S. lappa* showed considerable inhibitory activity. One of the active principles in the extract, namely **2**, seems to bind the cystein residues of tyrosine kinase and consequently inhibit the kinase activity in T-cell.[20]

Since the above-mentioned activity exhibited by **2** falls in the category of immunomodulators, it appeared worthwhile to undertake the synthesis of **2** and its congeners to correlate structure−activity relationships among these compounds. The present work was initiated with this aim in mind. It was realized that these compounds could be derived via a common cationic intermediate **I**, which in turn could be generated from mesylate (**4**) through solvolytic rearrangement.[21] In fact, **I** is presumed to be an intermediate during the biosynthesis of these compounds in nature (Figure 1).

Results and Discussion

The starting material used for the synthesis of key intermediate (11*S*)-1β-(mesyloxy)eudesm-4(14)-eno-13,6α-lactone (**4**) was the α,β-unsaturated ketone **6**, which was prepared from α-santonin (**5**) in six steps with an overall yield of 59%[3,22] (Scheme 1). Ketalization of **6** and subsequent isomerization of the double bond at C-2 by heating in ethylene glycol in the presence of TsOH at 145 °C gave a mixture of **7**, **8**, and **9** (70:1:0.3). Treatment of this mixture with boiling 50% AcOH−H_2O gave the desired β,γ-unsaturated ketone **10** in 64% overall yield. Dehydrogenation of **10** with DDQ in the presence of anhydrous TsOH at room temperature gave an *exo*-dienone **11** in 82% yield accompanied by an *endo*-dienone **12** in 7% yield. Treatment of **11** with zinc amalgam in refluxing AcOH gave γ,δ-unsaturated ketone **13** in 93% yield. Selective reduction of the C-1 carbonyl group of **13** with LiAl(*t*-BuO)$_3$H furnished the desired β-alcohol **14** and the corresponding α-alcohol **15** in 81% and 13% yields, respectively. The latter compound could be converted to **14** in 63% yield by Collins oxidation followed by reduction of the resulting ketone with LiAl(*t*-BuO)$_3$H. Mesylation of **14** with MsCl in pyridine at room temperature gave mesylate **4** in 97% yield. The *exo*-dienone **11** was also prepared by an alternative procedure. Dehydrogenation of **6** with a large excess of DDQ in the presence of anhydrous TsOH in refluxing toluene gave **11** in 57% yield based on recovered **6**.

Solvolytic rearrangement[21] of **4** with 0.5 M KOAc in AcOH provided a mixture (2:1:2.4) of tetra-, tri-, and disubstituted olefins **16**, **17**, and **1** in 63% yield. In addition, the solvolysis reaction also produced a mixture of olefins **18**, **19**, and **20** (5:4:7) in 16% yield, presumably formed by the epimerization of the exo-olefinic bond at C-4 to the thermodynamically more stable olefin during the reaction conditions employed. Separation of the crude reaction products by preparative HPLC gave compound **20**, along with a mixture (5:4) of **18** and **19**, and the compound **16**, along with a mixture (1:1.6:4) of **16**, **17**, and **1**. Since the

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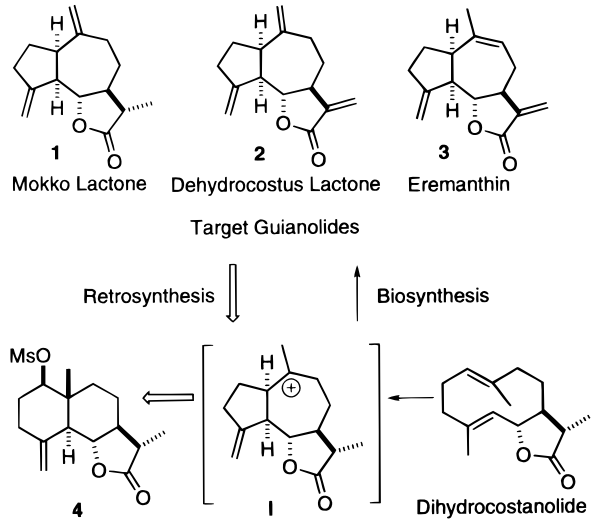


Figure 1. Retrosynthetic analyses and possible biosynthetic route of target guaianolides **1**−**3**.

tetrasubstituted olefin **16** was unstable, it was epoxidized to give a β-epoxide (**21**) and an α-epoxide (**22**) in 44% and 41% yields, respectively. Since the complete separation of **16**, **17**, and **1** by preparative HPLC was not achieved, the mixture of **16**, **17**, and **1** (1:1.6:4) was used as such for the next step.

Phenylselenenylation of the above-mentioned mixture with LDA and diphenyl diselenide[23] afforded a mixture of phenylseleno lactones, which was separated by HPLC to give **23**, together with a mixture of **24** and **25** (1:6), **26**, a mixture of **17** and **1** (1:4.3), and **27** in 7%, 25%, 12%, 6%, and 41% yields, respectively. The oxidative syn elimination[23,24] of **23** with 31% H_2O_2 gave an endo-unsaturated γ-lactone **28** in 87% yield. The physical constants and spectral data of **28** were in good agreement with those reported for 11,13-dihydro-7,11-dehydro-3-desoxyzaluzanin C.[25]

Treatment of the mixture (1:6) of **24** and **25** with 31% H_2O_2 furnished **3** and the corresponding endo-unsaturated γ-lactone (**29**) in 76% and 13% yields, respectively. The physical constants and spectral data of **3** were in good accordance with those of natural eremanthin reported in the literature.[8, 19]

Similarly, the oxidative syn elimination of **26** with 31% H_2O_2 gave an un-natural α-methylene γ-lactone **30** as an unstable compound. The structure of **30** was established with the help of spectral data including HREIMS. In a similar way, treatment of **27** with 31% H_2O_2 gave **2** in 99% yield. The physical constants and spectral data of **2** were in good agreement with those of natural dehydrocostus lactone reported in the literature.[17,18,20,26]

Selective epoxidation of the trisubstituted double bond of **17** using a mixture of **17** and **1** (1:4.3) gave an epoxide **31** and **1** in 13% and 48% yields, respectively. The compounds **1** and **31** were easily isolated in pure form from the crude reaction product by HPLC. The physical constants and spectral data of **1** were identical with those of natural mokko lactone reported in the literature.[17,20]

In conclusion, the natural products mokko lactone (**1**), dehydrocostus lactone (**2**), and eremanthin (**3**) have been synthesized from *l*-α-santonin in overall yields of 6.9% (13 steps), 4.8% (15 steps), and 2.2% (15 steps), respectively.

Having achieved the synthesis of the target compounds, it was of interest to prepare some derivatives to examine structure−activity relationships. Hence, we attempted syntheses of some α-methylene γ-lactone derivatives such as **33**, **37**, and **39** as depicted in Scheme 1.

Phenylselenenylation of **31** followed by treatment of the resulting phenylseleno lactone **32** with 31% H_2O_2 gave an α-methylene γ-lactone (**33**). Similarly, phenylselenenylation of β-epoxide **21** gave a mixture of α- and β-phenylseleno lactones **34** and **35** in 7% and 78% yields, respectively. The oxidative syn elimination of **34** and **35** with 31% H_2O_2 gave endo- and exo-unsaturated γ-lactones **36** and **37** in 86% and 85% yields, respectively. In an analogous manner, phenylselenenylation of **22** and successive treatment of phenylselenide **38** with 31% H_2O_2 gave an α-methylene γ-lactone **39** in 50% overall yield. Isodehydrocostus lactone (**40**) was also obtained from **20** by employing the same procedure.[27]

Inhibitory Activity of Killing Function of Cytotoxic T Lymphocytes. Cytotoxic T lymphocytes (CTL) are important in the elimination of virus-infected cells and tumors and in graft rejection in human body. Recently, we observed that dehydrocostus lactone (**2**) showed efficient inhibitory activity toward the killing function of CTL.[20] This result prompted us to examine the structure−activity relationships of **2** and the 14 related compounds that were synthesized as per the above-mentioned methods as inhibitors of killing function of CTL. The compounds were screened for inhibitors of cytotoxic activity of CTL clone OE4.

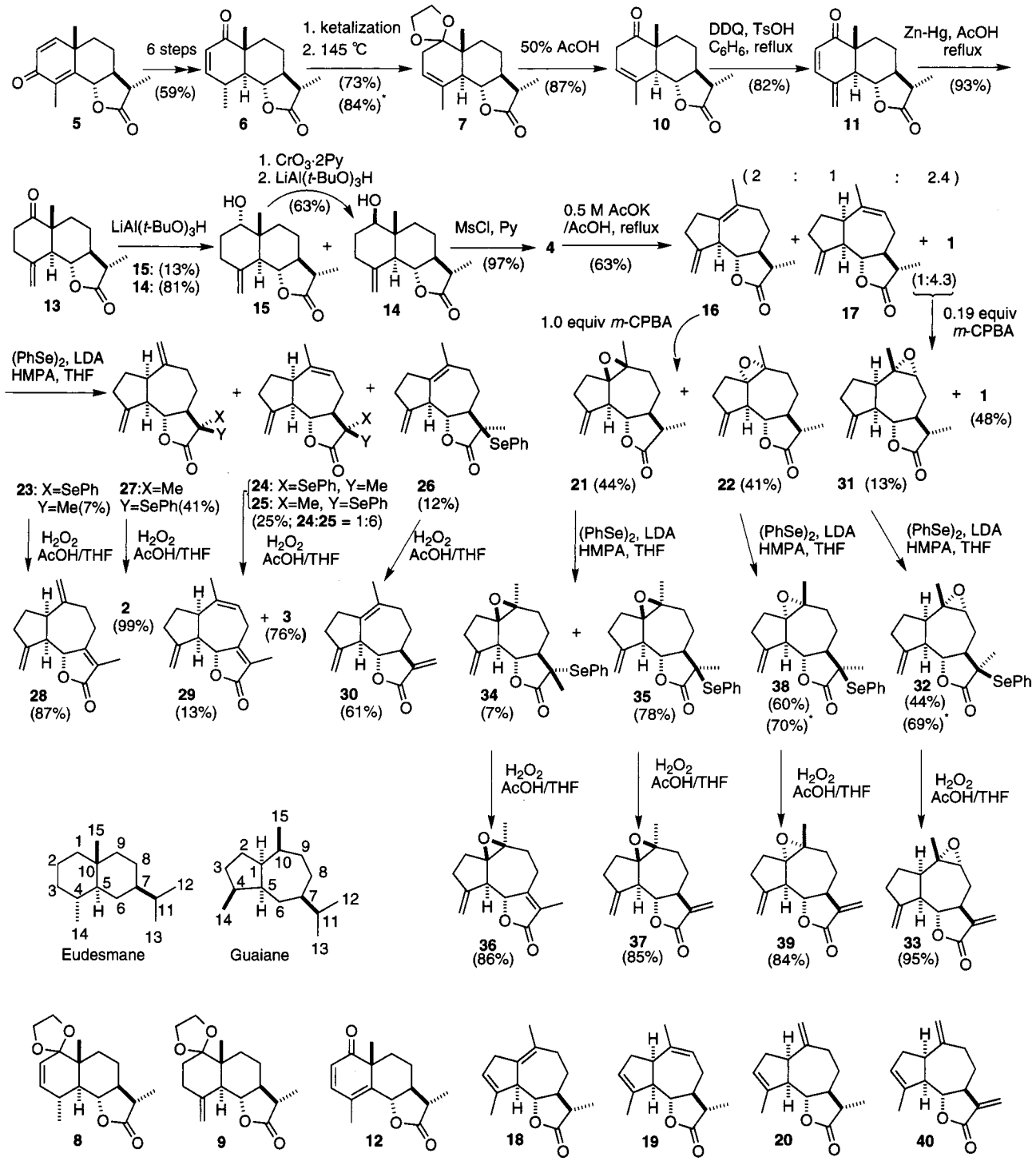
All compounds possessing an α-methylene γ-lactone moiety, such as dehydrocostus lactone (**2**), eremanthin (**3**), **30**, **33**, **37**, **39**, and isodehydrocostus lactone (**40**),[27] showed significant inhibitory activity on killing function of CTL. Hence, an α-methylene γ-lactone moiety in the molecule seems to be essential for the activity of these compounds. In fact, the activity of endo-unsaturated γ-lactones **28**, **29**, and **36** decreased markedly as compared with that of the corresponding α-methylene γ-lactones **2**, **3**, and **37**. It is noteworthy that α-methyl γ-lactones (saturated γ-lactones) such as isocostus lactone (**20**), mokko lactone (**1**), and other related compounds such as **21**, **22**, and **31** showed no significant activity (Table 1).

The positions of two double bonds in A and B rings of the molecule were not related in any way to the expression of bioactivity. Double-bond isomers such as dehydrocostus lactone (**2**), eremanthin (**3**), compound **30**, and isodehydrocostus lactone (**40**) showed approximately the same activity. It was observed that the magnitude of inhibitory activity was influenced by replacing the double bond in the B ring with an epoxide ring. Introduction of the epoxide moiety enhanced the inhibitory activity.

In summary, it has been conclusively established during the present study that the compounds **33**, **37**, and **39** showed promising inhibitory activity on the killing function of cytotoxic T lymphocytes, and the order of the inhibitory activity in the test samples investigated was **33** = **37** = **39** ≥ **3** ≥ **2** ≥ **30** ≥ **40** > **28** > **20**, **29** > **31** ≥ **21** > **1**, **22**, **36**.

Inhibitory Activity on Expression of Intercellular Adhesion Molecule-1 (ICAM-1) Induced by Interleukin-1. Expression of intercellular adhesion molecule-1 (ICAM-1) is induced by interleukin-1 (IL-1) on the surface of endothelial cells of blood vessels. ICAM-1 on the activated endothelial cells interacts with lymphocyte function-associated antigen-1 (LFA-1) on leucocytes in the blood stream, and the leucocytes begin rolling, adhere to the surface of endothelium, and finally migrate from the inside of the blood vessel to the inflammatory portion by chemotaxis. The attack of leucocytes causes serious damage to the inflammatory tissue. Expression of excess amount of ICAM-1 on the surface of endothelial cells of a blood vessel plays an important role in the progress of inflammatory

Scheme 1[a]



[a] The yields are based on the recovered starting material.

Table 1. Inhibitory Activity of Killing Function of Cytotoxic T Lymphocytes (CTL)[a]

	α-methylene γ-lactones (exo-unsaturated γ-lactones)							endo-unsaturated γ-lactones			α-methyl γ-lactones (saturated γ-lactones)				
compounds	**30**	**3**	**2**	**33**	**37**	**39**	**40**	**28**	**29**	**36**	**20**	**1**	**21**	**22**	**31**
IC_{50} (μM)	15	11	14	8	7	7	16	50	>100	>1000	>100	>1000	597	>1000	422

[a] To examine the dose response of the compounds, OE4 (1×10^4 cells/well) was preincubated with various concentrations of the compounds for 2 h, mixed with P815 (1×10^3 cells/well) labeled with [^{3}H]thymidine, and incubated for 4 h. The experiments were carried out in triplicate cultures. IC_{50} was deduced from the 50% inhibition of the specific release, which was calculated by using the formula described in the Materials and Methods for Bioassays.

reaction. These facts suggest that the inhibitors of induction of ICAM-1 may turn out to yield a new type of antiinflammatory agent. With this in mind, we began to examine **2** and the 12 related compounds that were synthesized by us for determining their inhibitory activity on the induction of ICAM-1 through bioassay.

Table 2. Inhibitory Activity of Induction of Intercellular Adhesion Molecule-1 (ICAM-1)[a]

	α-methylene γ-lactones (exo-unsaturated γ-lactones)						endo-unsaturated γ-lactones			α-methyl γ-lactones (saturated γ-lactones)			
compounds	**30**	**3**	**2**	**33**	**37**	**39**	**28**	**29**	**36**	**1**	**21**	**22**	**31**
IC_{50} (μM)	8	46	10	6	10	7	442	142	136	251	504	253	117

[a] A549 (3×10^4 cells/well) was pretreated with various concentrations of the compounds for 1 h and then incubated with the addition of IL-1β for 6 h. Absorbance of 415 nm was assayed after treatment of the cells with primary and secondary antibodies and addition of the enzyme substrate as described in the Materials and Methods for Bioassays. The experiments were carried out in triplicate cultures. IC_{50} was calculated by using the formula in Materials and Methods for Bioassays.

The above-mentioned compounds were screened for inhibition of induction of ICAM-1 using culture cells A 549 (lung carcinoma, ATCC CCL 185), an in vitro model of human endothelial cell. All compounds possessing an α-methylene γ-lactone moiety, such as dehydrocostus lactone (**2**), eremanthin (**3**), **30**, **33**, **37**, and **39**, showed significant inhibitory activity on induction of ICAM-1. Hence, it is inferred that an α-methylene γ-lactone moiety in the molecule must be essential for activity in the case of these compounds. In fact, the α-methylene γ-lactone (**37**) showed 14-fold stronger activity than the corresponding endo-unsaturated γ-lactone (**36**) and 50-fold stronger activity than the corresponding saturated α-methyl γ-lactone (**21**) (Table 2).

The extent of inhibitory activity expressed was influenced by the location of the double bond in the B-ring and by the introduction of an epoxide ring at the C-9 or C-1(10) positions. Thus, the epoxide **33** showed 8-fold stronger activity than that of the corresponding unsaturated compound, eremanthin (**3**). The most active compound obtained was the α-methylene γ-lactone derivative **33**, and the order of the inhibitory activity exhibited in the test samples was **33** ≥ **39** ≥ **30** ≥ **37** = **2** > **3** > **31** > **36** ≥ **29** > **1** = **22** > **28** > **21**.

In our preliminary results, it appears that these compounds inhibit induction of ICAM-1 by controlling the nuclear transportation of NF-κB, which is a transcription factor of ICAM-1 gene. It is interesting to note that the possible target of compounds in inhibition of the ICAM-1 induction may be different from those in the inhibition of the killing function of CTL. Details regarding the mode of action of these compounds will be reported elsewhere.

Experimental Section

General Experimental Procedures. All melting points are uncorrected. ^{1}H NMR spectra were recorded at 200 MHz in $CDCl_3$, and ^{13}C NMR spectra were recorded at 50 MHz in $CDCl_3$ unless otherwise stated. The ^{1}H NMR assignments were determined by decoupling and H−H COSY experiments. The ^{13}C NMR assignments were determined by DEPT and C−H COSY experiments or DEPT, HMQC, and HMBC experiments (at 125.7 MHz). Reactions were run under an atmosphere of N_2 or Ar. THF was distilled from sodium benzophenone ketyl. $CHCl_3$ was distilled from CaH_2. Silica gel (70−200 mesh) was employed for column chromatography and silica gel (230−400 mesh) for flash column chromatography. To describe HPLC conditions, we designate column, solvent, flow rate (mL/min), and retention time (t_R (min)) as per following order. The column codes are as follows: A, 250- × 4.6-mm i.d. stainless column packed with 10-mm silica gel; B, 250- × 8-mm i.d. stainless column packed with 10-mm silica gel; C, 300- × 10-mm i.d. glass column packed with 10-mm silica gel; D, 500- × 30-mm i.d. stainless column packed with 25−40-mm silica gel.

(11*S*)-1,1-(Ethylenedioxy)eudesm-3-eno-13,6α-lactone (7). A mixture of **6** (2.30 g, 9.26 mmol), ethylene glycol (137 mL), and $TsOH \cdot H_2O$ (305 mg, 1.60 mmol) in dry benzene (500 mL) was refluxed in a flask equipped with a Dean−Stark column packed with molecular sieves for 24 h, and then benzene was removed. The residue was heated at 145−150 °C for 22 min, cooled, poured into a saturated aqueous solution of NaCl (250 mL), and extracted with EtOAc (3 × 150 mL). The combined extracts were washed successively with saturated aqueous NaCl (3 × 30 mL), dried (Na_2SO_4), and concentrated to give a crude product (2.94 g), which was chromatographed over silica gel [195 g, 5.5-cm i.d., EtOAc−hexane (2:8)].

The first elution gave a mixture (70:1:0.3) of compounds **7**−**9** (1.97 g, 73%), which was employed without further purification for the next reaction. A part of this mixture was recrystallized from ether to give **7** as colorless prisms: mp 124−126 °C; $[\alpha]^{20}_D$ +105.1° (*c* 1.25, $CHCl_3$); IR ($CHCl_3$) ν_{max} 1770 cm^{-1}; ^{1}H NMR δ 1.03 (3H, s, H-15), 1.22 (3H, d, J = 6.8 Hz, H-12), 1.86 (3H, br s, $W_{h/2}$ = 5.0 Hz, H-14), 2.69 (1H, br d, J = 11.5 Hz, H-5), 3.75−4.18 (5H, m, −O(CH$_2$)$_2$O−, H-6), 5.32 (1H, br s, $W_{h/2}$ = 10.0 Hz, H-3); ^{13}C NMR δ 12.39 (q, C-12), 15.75 (q, C-15), 22.54, 28.38 (t, C-8 and C-9), 23.50 (q, C-14), 34.49 (t, C-2), 40.57 (d, C-11), 43.84 (s, C-10), 48.43 (d, C-5), 53.23 (d, C-7), 64.81, 65.49 (t, −OCH$_2$CH$_2$O−), 81.37 (d, C-6), 110.88 (s, C-1), 120.42 (d, C-3), 133.99 (s, C-4), 179.58 (s, C-13); *anal*. C 69.70%, H 8.27%, calcd for $C_{17}H_{24}O_4$, C 69.83%, H 8.27%.

The second elution gave recovered **6** (304 mg, 13%). Although the mixture of compounds **7**−**9** appeared as a broad single peak in HPLC [A, EtOAc−hexane (2:8), 3.0 mL/min], the pure compounds **8** and **9** were isolated by repeated HPLC, using the mother liquor of **7**, by following the above-mentioned conditions.

Compound **8** was obtained as colorless needles (EtOAc): mp 152 °C; $[\alpha]^{20}_D$ +75.4° (*c* 0.68, $CHCl_3$); IR ($CHCl_3$) ν_{max} 1777 cm^{-1}; ^{1}H NMR δ 1.06 (3H, s, H-15), 1.22 (6H, d, J = 6.8 Hz, H-12, H-14), 2.14−2.34 (2H, m, H-4, 11), 3.42−4.20 (4H, m, −O(CH$_2$)$_2$O−), 3.88 (1H, dd, J = 10.7, 10.7 Hz, H-6), 5.50 (1H, dd, J = 10.0, 2.1 Hz, H-3), 5.61 (1H, dd, J = 10.0, 2.2 Hz, H-2); ^{13}C NMR δ 12.40 (q, C-12), 15.97 (q, C-15), 20.55 (q, C$_{14}$), 23.09 (t), 29.52 (t), 32.80 (d), 40.83 (d), 43.44 (s, C-10), 48.29 (d, C-5), 52.00 (d, C-7), 65.11, 65.16 (t, −OCH$_2$CH$_2$O−), 84.28 (d, C-6), 108.93 (s, C-1), 125.45 (d, C-3), 135.76 (d, C-2), 179.45 (s, C-13); *anal*. C 69.74%, H 8.28%, calcd for $C_{17}H_{24}O_4$, C 69.83%, H 8.27%.

Compound **9** was obtained as colorless needles (EtOAc): mp 129 °C; $[\alpha]^{20}_D$ +135.4° (*c* 1.09, $CHCl_3$); IR ($CHCl_3$) ν_{max} 1772, 1652 cm^{-1}; ^{1}H NMR δ 0.95 (3H, s, H-15), 1.19 (3H, d, J = 6.8 Hz, H-12), 2.59 (1H, J = 11.0 Hz, H-5), 3.80−4.06 (5H, m, −O(CH$_2$)$_2$O−, H-6), 4.80 (1H, s, H-14a), 4.94 (1H, s, H-14b); ^{13}C NMR δ 12.42 (q, C-12), 15.91 (q, C-15), 22.73 (t), 28.94 (t), 31.27 (t), 32.43 (t), 41.05 (d, C-11), 45.94 (s, C-10), 49.96 (d, C-5), 51.74 (d, C-7), 65.12, 65.22 (t, −OCH$_2$CH$_2$O−), 79.63 (d, C-6), 110.00 (t, C-14), 111.52 (s, C-1), 143.37 (s, C-4), 179.40 (s, C-13); *anal*. C 70.03%, H 8.34%, calcd for $C_{17}H_{24}O_4$, C 69.83%, H 8.27%.

(11*S*)-1-Oxoeudesm-3-eno-13,6α-lactone (10). A solution of the mixture (70:1:0.3) of ketals **7**−**9** (3.65 g, 12.5 mmol) in an aqueous solution of acetic acid (260 mL) was refluxed for 22 min. The mixture was cooled, poured into a saturated aqueous solution of NaCl (300 mL), and extracted with EtOAc (3 × 100 mL). The combined extracts were worked up to give a crude product, which was purified by the combination of column chromatography [silica gel 130 g, 5.5-cm i.d., EtOAc−hexane (2:8, 3:7, and 1:1)] and HPLC [B, EtOAc−hexane (2:8), 9.9 mL/min] affording a mixture (38:1) of desired compounds **10** and **13** (2.79 g, 87%), which was employed as such for the next reaction. Slow elution of column chromatography gave the α,β-unsaturated ketone **6** (105 mg, 3%).

The mixture of **10** and **13** was recrystallized from EtOAc to give **10** as colorless needles: mp 138−139 °C; $[\alpha]^{20}_D$ +61.1° (*c* 1.74, $CHCl_3$); IR ($CHCl_3$) ν_{max} 1774, 1714 cm^{-1}; ^1H NMR δ 1.13 (3H, s, H-15), 1.24 (3H, d, J = 6.9 Hz, H-12), 1.96 (3H, br s, $W_{h/2}$ = 5.0 Hz, H-14), 2.32 (1H, dq, J = 12.3, 6.7 Hz, H-11), 2.60 (1H, br d, J = 11.2 Hz, H-5), 2.84 (1H, ddd, J = 22.0, 5.2, 2.6 Hz, H-2ax), 3.01 (1H, ddd, J = 22.0, 4.8, 1.6 Hz, H-2eq), 4.09 (1H, dd, J = 11.2, 9.7 Hz, H-6), 5.52−5.60 (1H, m, H-3); ^{13}C NMR δ 12.40 (q, C-12), 16.85 (q, C-15), 22.13 (q, C-14), 22.13 (t), 31.64 (t), 38.33 (t, C-2), 40.51 (d, C-11), 48.10 (s, C-10), 48.25 (d, C-5), 52.74 (d, C-7), 80.25 (d, C-6), 119.18 (d, C-3), 135.52 (s, C-4), 179.04 (s, C-13), 212.24 (s, C-1); *anal.* C 72.47%, H 8.04%, calcd for $C_{15}H_{20}O_3$, C 72.55%, H 8.12%.

(11*S*)-1-Oxoeudesm-2,4(14)-dieno-13,6α-lactone (11). A mixture of $TsOH \cdot H_2O$ (1.97 g, 10.4 mmol) and benzene (70 mL) was refluxed in a flask equipped with a Dean−Stark column packed with molecular sieves to eliminate H_2O for 1.5 h, and then the mixture (38:1) of **10** and **13**, together with DDQ (1.94 g, 8.29 mmol), and benzene (40 mL) was added at 25 °C. After being stirred for 20 min, the mixture was filtered through Celite, and the filtrate was poured into a saturated aqueous solution of NaCl (150 mL) and extracted with EtOAc (3 × 50 mL). The combined extracts were worked up to give spectroscopically pure **11** (935 mg, 55%) as a crystalline solid. The mother liquor was purified by column chromatography [silica gel, 56 g; 3.3-cm i.d., EtOAc−hexane (2:8)] and HPLC [B, EtOAc−hexane (2:8), 9 mL/min; t_R: **11**, 5.6 min; **12**, 7.6 min] to give spectroscopically pure **11** (465 mg, 27%) and **12** (125 mg, 7%). The combined yield of compound **11** was 82%.

Compound **11** was obtained as colorless needles (EtOAc): mp 110−113 °C; $[\alpha]^{20}_D$ +389.9° (*c* 0.70, $CHCl_3$); IR ($CHCl_3$) ν_{max} 1782, 1678 cm^{-1}; ^1H NMR δ 1.08 (3H, s, H-15), 1.26 (3H, d, J = 6.9 Hz, H-12), 2.35 (1H, dq, J = 12.1, 6.9 Hz, H-11), 2.83 (1H, br d, J = 10.4 Hz, H-5), 4.17 (1H, dd, J = 10.4, 10.4 Hz, H-6), 5.54 (1H, d, J = 1.8 Hz, H-14a), 5.82 (1H, d, J = 1.8 Hz, H-14b), 5.87 (1H, d, J = 9.9 Hz, H-2), 7.05 (1H, d, J = 9.9 Hz, H-3); ^{13}C NMR δ 12.36 (q, C-12), 17.74 (q, C-15), 22.35 (t), 31.47 (t), 40.58 (d, C-11), 47.17 (s, C-10), 49.49 (d, C-5), 51.91 (d, C-7), 79.35 (d, C-6), 122.06 (t, C-14), 125.13 (d, C-2), 139.71 (s, C-4), 146.51 (d, C-3), 178.82 (s, C-13), 202.34 (s, C-1); *anal.* C 73.08%, H 7.57%, calcd for $C_{15}H_{18}O_2$, C 73.14%, H 7.37%.

Compound **12** was obtained as colorless needles (EtOAc): mp 142−145 °C; $[\alpha]^{20}_D$ −102.3° (*c* 0.87, $CHCl_3$); IR ($CHCl_3$) ν_{max} 1788, 1670, 1638 cm^{-1}; ^1H NMR δ 1.26 (3H, d, J = 6.9 Hz, H-12), 1.34 (3H, s, H-15), 2.17 (3H, d, J = 1.8 Hz, H-14), 2.37 (1H, dq, J = 11.9 Hz, 6.9, H-11), 4.72 (1H, br d, J = 10.4 Hz, H-6), 6.04 (1H, d, J = 9.8 Hz, H-2), 6.84 (1H, d, J = 9.8 Hz, H-3); ^{13}C NMR δ 12.31 (q, C-12), 18.95 (q, C-15), 23.23 (t), 26.10 (q, C-14), 35.33 (t), 40.91 (d, C-11), 50.41 (s, C-10), 53.05 (d, C-7), 81.62 (d, C-6), 121.29 (s, C-5), 123.38 (d, C-2), 143.04 (s, C-4), 148.93 (d, C-3), 177.92 (s, C-13), 204.40 (s, C-1); *anal.* C 73.14%, H 7.47%, calcd for $C_{15}H_{18}O_3$, C 73.14%, H 7.37%.

Alternate Route for the Preparation of 11. Dehydrogenation of 6 with DDQ. A solution of anhydrous TsOH (139 mg), **6** (100 mg, 0.40 mmol), and DDQ (187 mg, 0.80 mmol) in toluene (3 mL) was refluxed for 7 h. During this period, more DDQ (94.3 mg, 0.40 mmol) was added into the reaction mixture in two lots. After cooling, the reaction mixture was filtered through Celite under reduced pressure. The filtrate was worked up to give a crude oily product (112 mg), which was purified by the combination of column chromatography [silica gel, 8 g; 1.8-cm i.d., EtOAc−hexane (2:8)] and HPLC [A, EtOAc−hexane (2:8), 3 mL/min] to give **11**(t_R 6.0 min, 42 mg, 41%) and recovered **6** (t_R 7.2 min, 29 mg, 28%).

(11*S*)-1-Oxoeudesm-4(14)-eno-13,6α-lactone (13). A mixture of **11** (495 mg, 2.01 mmol) and Zn−Hg[28] in AcOH (13 mL) was refluxed for 1 h under Ar and filtered. The filtrate was poured into a saturated aqueous solution of NaCl (30 mL) and extracted with EtOAc (4 × 10 mL). The combined extracts were worked up to give spectroscopically pure **13** (409 mg, 82%) as colorless needles. The mother liquor was subjected to the combination of column chromatography [silica gel, 3.6 g; 1.3-cm i.d., EtOAc−hexane (2:8)] and HPLC [A; EtOAc−hexane (2:8), 3.0 mL/min] to give **13** (55 mg, 11%). The combined yield of **13** was 93%. Compound **13** was obtained as colorless needles (EtOAc): mp 154−155 °C; $[\alpha]^{20}_D$ +154.7° (*c* 0.60, $CHCl_3$); IR ($CHCl_3$) ν_{max} 1782, 1710 cm^{-1}; ^1H NMR δ 1.13 (3H, s, H-15), 1.24 (3H, d, J = 6.9 Hz, H-12), 4.12 (1H, dd, J = 10.4, 10.4 Hz, H-6), 5.08 (1H, s, H-14a), 5.21 (1H, s, H-14b); ^{13}C NMR δ 12.45 (q, C-12), 18.12 (q, C-15), 22.49 (t), 31.47 (t), 33.98 (t, C-2), 37.60 (t, C-3), 41.02 (d, C-11), 50.23 (s, C-10), 51.78 (d, C-7), 52.22 (d, C-5), 78.58 (d, C-6), 112.63 (t, C-14), 140.75 (s, C-4), 178.87 (s, C-13), 211.99 (s, C-1); *anal.* C 72.37%, H 8.25%, calcd for $C_{15}H_{20}O_3$, C 72.55%, H 8.25%.

(11*S*)-1β-Hydroxyeudesm-4(14)-eno-13,6α-lactone (14) and (11*S*)-1α-Hydroxyeudesm-4(14)-eno-13,6α-lactone (15). To a stirred solution of **13** (1.14 g, 4.58 mmol) in THF (20 mL) at 0 °C was added $LiAl(t\text{-}BuO)_3H$ (4.67 g, 18.32 mmol). The mixture was stirred for 1 h at 0 °C, 2 M HCl (20 mL) was added, and stirring was continued for 10 min at 0 °C. The mixture was then filtered through Celite under reduced pressure. The filtrate was poured into a saturated aqueous solution of NaCl (200 mL) and extracted with EtOAc (3 × 60 mL). The combined extracts were worked up as usual to give a pale yellow oil (1.21 g), which was purified by preparative HPLC [D, EtOAc−hexane (3:7), 9.0 mL/min] to give **14** (t_R 7.8 min, 843 mg, 73%) and **15** (t_R 5.4 min, 98 mg, 9%). The mixture of **14** and **15** (181 mg) obtained as the middle fraction in the above-mentioned preparative HPLC was further purified by another HPLC [B, EtOAc−hexane (3:7), 10 mL/min] to give **14** (t_R 6.4 min, 86.7 mg, 8%) and **15** (t_R 4.0 min, 51 mg, 4%). The total yields of **14** and **15** were 930 mg (81%) and 149 mg (13%), respectively.

Compounds **14** was obtained as colorless needles (EtOAc): mp 145−146 °C; $[\alpha]^{20}_D$ +130.45° (*c* 0.892, $CHCl_3$), IR ($CHCl_3$) ν_{max} 3520, 1774 cm^{-1}; ^1H NMR δ 0.83 (3H, s, H-15), 1.23 (3H, d, J = 6.9 Hz, H-12), 2.11 (1H, br d, J = 10.4 Hz, H-5), 3.50 (1H, dd, J = 11.5, 4.7 Hz, H-1), 4.05 (1H, dd, J = 10.4, 10.4 Hz, H-6), 4.83 (1H, d, J = 1.4 Hz, H-14a), 4.97 (1H, d, J = 1.4 Hz, H-14b); ^{13}C NMR δ 11.59 (q, C-12), 12.45 (q, C-15), 22.95 (t), 31.18 (t), 33.46 (t, C-3), 35.89 (t, C-2), 41.13 (d, C-11), 42.79 (s, C-10), 52.25 (d, C-7), 52.40 (d, C-5), 78.15 (d, C-1), 79.29 (d, C-6), 110.23 (t, C-14), 142.75 (s, C-4), 179.36 (s, C-13); *anal.* C 71.87%, H 8.87%, calcd for $C_{15}H_{22}O_3$, C 71.97%, H 8.86%.

Compound **15** was obtained as colorless needles (EtOAc): mp 146−148 °C; $[\alpha]^{20}_D$ +166.5° (*c* 0.92, $CHCl_3$), IR ($CHCl_3$) ν_{max} 3520, 1774 cm^{-1}; ^1H NMR δ 0.86 (3H, s, H-15), 1.22 (3H, d, J = 6.9 Hz, H-12), 2.70 (1H, d, J = 11.4 Hz, H-5), 3.46 (1H, dd, J = 2.6, 2.6 Hz, H-1), 4.03 (1H, dd, J = 11.4, 10.1 Hz, H-6), 4.80 (1H, d, J = 1.5 Hz, H-14a), 4.95 (1H, d, J = 1.5 Hz, H-14b); ^{13}C NMR δ 12.42 (q, C-15), 18.18 (q, C-12), 22.90 (t), 29.90 (t), 30.07 (t), 33.20 (t), 41.08 (d, C-11), 42.71 (s, C-10), 47.03 (d, C-5), 51.95 (d, C-7), 74.18 (d, C-1), 80.06 (d, C-6), 108.91 (t, C-14), 144.58 (s, C-4), 179.61 (s, C-13); *anal.* C 71.85%, H 8.74%, calcd for $C_{15}H_{22}O_3$, C 71.97%, H 8.86%.

Oxidation of 15 with $CrO_3 \cdot 2Py$. Formation of 13 from 15. CrO_3 (720 mg, 7.2 mmol) was added to a mixture of CH_2Cl_2 (6 mL) and pyridine (1.16 mL, 14.4 mmol) at 0 °C and stirred for 10 min. Then, **15** (90 mg, 0.36 mmol) dissolved in CH_2Cl_2 (4 mL) was added over 5 min. The mixture was stirred at 0 °C for 3 h and then at room temperature for 2 h and filtered through Celite under reduced pressure. The filtrate was worked up to give a crystalline crude product of **13** (79 mg), which was subsequently purified by HPLC [C, EtOAc−hexane (3:7), 3.0 mL/min] to give **13** (69 mg, 78%) as colorless needles (EtOAc): mp 155 °C.

(11*S*)-1β-(Mesyloxy)eudesm-4(14)-eno-13,6α-lactone (4). To a stirred solution of **14** (1.20 g, 4.80 mmol) in pyridine (50 mL) was added MsCl (533 μL, 7.20 mmol). The mixture was stirred for 3.5 h at 25 °C and worked up to give spectroscopically pure **4** (1.53 g, 97%). Compound **4** was obtained as colorless prisms (ether): mp 122−124 °C; $[\alpha]^{20}_D$ +98.9° (*c* 0.27, $CHCl_3$); IR ($CHCl_3$) ν_{max} 1774, 1338, 1174 cm^{-1}; ^1H NMR δ 0.92 (3H, s, H-15), 1.23 (3H, d, J = 6.8 Hz, H-12), 3.03 (3H, s, −OMs), 4.02 (1H, dd, J = 10.4, 10.4 Hz, H-6), 4.57 (1H, dd, J =11.5, 4.7 Hz, H-1), 4.89 (1H, d, J = 1.3 Hz, H-14a), 5.04 (1H, d, J = 1.3 Hz, H-14b); ^{13}C NMR δ 12.35 (q, C-15), 12.44 (q, C-12), 22.60 (t), 28.98 (t), 32.88 (t), 35.83 (t), 38.90 (q, −OMs), 40.90 (d, C-11), 42.08 (s, C-10), 51.91 (d, C-7), 52.29 (d, C-5),

78.48 (d, C-6), 87.66 (d, C-1), 111.43 (t, C-14), 140.98 (s, C-4), 178.89 (s, C-13); HREIMS *m/z* 328.1340 (calcd for $C_{16}H_{24}O_5S$ 328.1345).

Solvolysis of 4. Formation of (11*S*)-Guaia-1(10),4(14)-dieno-13,6α-lactone (16), (11*S*)-Guaia-4(14),9-dieno-12,6α-lactone (17), and (11*S*)-Guaia-4(14),10(15)-dieno-13,6α-lactone (1). A mixture of **4** (1.49 g, 4.55 mmol) and 0.5 M KOAc in AcOH (120 mL) was refluxed under stirring for 22 h, cooled, poured into a saturated aqueous solution of NaCl (150 mL), and extracted with EtOAc (3 × 50 mL). The combined extracts were worked up to give a crude oily material (1.06 g) which was passed through short column of silica gel [40 g; 3-cm i.d., EtOAc−hexane (1:9)]. The eluent was further purified by HPLC [C, EtOAc−hexane (5:95), 9.0 mL/min].

The first peak (t_R 4.0 min) gave a mixture (5:4) of (11*S*)-guaia-1(10),3-dieno-13,6α-lactone (**18**) and (11*S*)-guaia-3,9-dieno-13,6α-lactone (**19**) (73.1 mg, 7%). A part of this mixture was purified by repeated HPLC to give **18** as colorless needles (EtOAc): mp 80 °C; $[\alpha]^{25}_D$ +8.9° (*c* 0.41, $CHCl_3$); IR ($CHCl_3$) ν_{max} 1762 cm^{-1}; ^1H NMR δ 1.22 (3H, d, *J* = 6.9 Hz, H-12), 1.72 (3H, br s, H-15), 1.91 (3H, br s, H-14), 2.96 (2H, br s, H-2), 3.30 (1H, br d, *J* = 9.9 Hz, H-5), 3.66 (1H, dd, *J* = 9.9, 9.9 Hz, H-6), 5.52 (1H, br s, H-3); *anal*. C 77.03%, H 8.65%, calcd for $C_{15}H_{20}O_2$, C 77.55%, H 8.68%. **19:** ^1H NMR (based on the mixture of **18** and **19**) δ 1.23 (3H, d, *J* = 7.0 Hz, H-12), 1.79 (3H, br s, H-15), 1.91 (3H, br s, H-14), 4.03 (1H, dd, *J* = 9.3, 9.3 Hz, H-6), 5.46 (1H, br d, *J* = 7.7 Hz, H-9), 5.52 (1H, br s, H-3).

The second peak (t_R 5.6 min) gave (11*S*)-guaia-3,10(15)-dieno-12,6α-lactone (**20**) (95.1 mg, 9%) as a pale yellow oil: $[\alpha]^{25}_D$ +71.4° (*c* 0.64, $CHCl_3$); IR ($CHCl_3$) ν_{max} 1760, 1640, 902 cm^{-1}; ^1H NMR δ 1.24 (3H, d, *J* = 6.8 Hz, H-12), 1.83 (3H, br s, H-14), 2.78 (1H, br dd, *J* = 9.0, 9.7 Hz, H-5), 3.10 (1H, q, *J* = 7.0 Hz, H-1), 3.98 (1H, dd, *J* = 9.7, 9.7 Hz, H-6), 4.84 (1H, d, *J* = 1.7 Hz, H-15), 4.87 (1H, d, *J* = 1.7 Hz, H-15), 5.53 (1H, br s, H-3); HREIMS *m/z* 232.1450 (calcd for $C_{15}H_{20}O_2$ 232.1463).

The third peak gave **16** (169.1 mg, 16%) as a highly unstable viscous oil: IR ($CHCl_3$) ν_{max} 1770 cm^{-1}; ^1H NMR δ 1.23 (3H, d, *J* = 6.9 Hz, H-12), 1.75 (3H, s, H-15), 3.24 (1H, br dd, *J* = 10.1, 1.2 Hz, H-5), 3.65 (1H, dd, *J* = 9.9, 9.9 Hz, H-6), 5.08 (1H, d, *J* = 1.2 Hz, H-14a), 5.13 (1H, d, *J* = 1.2 Hz, H-14b).

The fourth peak gave a mixture (1:1.6:4) of **16**, **17**, and **1** (496.8 mg, 47%), which was employed for the next reaction without further purification. **17**: ^1H NMR (scanned on the total mixture of **16**,**17**, and **1**) δ 1.23 (3H, d, *J* = 7.0 Hz, H-12), 1.82 (3H, s, H-15), 3.95 (1H, dd, *J* = 9.8, 9.8 Hz, H-6), 5.52 (1H, br d, *J* = 7.7 Hz, H-9). Repeated HPLC of the mixture gave pure Mokko Lactone (**1**) as a colorless oil: $[\alpha]^{20}_D$ +26.9° (*c* 0.31, $CHCl_3$); IR ($CHCl_3$) ν_{max} 1744, 1676 cm^{-1}; ^1H NMR (500 MHz) δ 1.24 (3H, d, *J* = 7.0 Hz, H-12), 1.30 (1H, dddd, *J* = 12.1, 12.1, 11.7, 5.1 Hz, H-8), 1.86 (1H, dddd, *J* = 17.2, 10.3, 4.5, 1.5 Hz, H-2), 1.95 (2H, m, H-2, H-7), 2.05 (1H, ddd, *J* = 12.1, 12.1, 5.1 Hz, H-9), 2.11 (1H, dddd, *J* = 12.5, 10.3, 5.0, 1.7 Hz, H-3), 2.21 (1H, dq, *J* = 11.7, 7.0 Hz, H-11), 2.52 (3H, m, H-3, H-8, H-9), 2.80 (1H, dd, *J* = 9.4, 8.0 Hz, H-5), 2.88 (1H, ddd, *J* = 8.0, 8.0, 4.5 Hz, H-1), 3.92 (1H, dd, *J* = 9.4, 9.4 Hz, H-6), 4.78 (1H, br s, $W_{h/2}$ = 3.0 Hz, H-15a), 4.88 (1H, br s, $W_{h/2}$ = 3.0 Hz, H-15b), 5.05 (1H, d, *J* = 2.2 Hz, H-14a), 5.20 (1H, d, *J* = 2.0 Hz, H-14b); ^{13}C NMR (125 MHz) δ 13.23 (q, C-12), 30.18 (t, C-2), 32.49 (t, C-3, C-8), 37.64 (t, C-9), 42.05 (d, C-11), 47.07 (d, C-1), 49.88 (d, C-7), 51.95 (d, C-5), 85.25 (d, C-6), 109.17 (t, C-14), 111.82 (t, C-15), 149.94 (s, C-10), 151.68 (s, C-4), 178.66 (s, C-13); HREIMS *m/z* 232.1456 (calcd for $C_{15}H_{20}O_2$ 232.1463).

Epoxidation of 16 with *m*-CPBA. Formation of (11*S*)-1β,10β-Epoxyguai-4(14)-eno-13,6α-lactone (21) and (11*S*)-1α,10α-Epoxyguai-4(14)-eno-13,6α-lactone (22). A solution of **16** (134.0 mg, 0.577 mmol) in $CHCl_3$ (12 mL) was stirred with 85% *m*-CPBA (117.1 mg, 0.577 mmol) for 1 h at 0 °C, poured into a mixture of a 0.1 M aqueous solution of KI (15 mL) and a saturated aqueous solution of NaCl (10 mL), and extracted with $CHCl_3$ (4 × 10 mL). The combined extracts were washed with a 0.1 M aqueous solution of $Na_2S_2O_3$ (3 × 8 mL) and a saturated aqueous solution of NaCl (3 × 8 mL), dried (Na_2SO_4), and concentrated to give a crude oily product (175 mg), which was purified by the combination of column chromatography [silica gel, 12 g; 2.0-cm i.d., EtOAc−hexane (2:8)] and HPLC [A, EtOAc−hexane (2:8), 3.0 mL/min]. The first peak (t_R 3.2 min) gave spectroscopically pure **21** (62.6 mg, 44%) as colorless needles (EtOAc): mp 76−78 °C; $[\alpha]^{20}_D$ +20.8° (*c* 0.46, $CHCl_3$); IR ($CHCl_3$) ν_{max} 1772 cm^{-1}; ^1H NMR δ 1.19 (3H, d, *J* = 6.9 Hz, H-12), 1.36 (3H, s, H-15), 2.82 (1H, dd, *J* = 10.0, 2.5 Hz, H-5), 3.99 (1H, dd, *J* = 10.0, 9.8 Hz, H-6), 5.12 (1H, d, *J* = 1.9 Hz, H-14a), 5.41 (1H, d, *J* = 2.1 Hz, H-14b); ^{13}C NMR δ 12.35 (q, C-12), 22.94 (q, C-15), 22.94 (t), 31.08 (t), 32.41 (t), 34.19 (t), 41.08 (d, C-11), 49.03 (d, C-5), 53.40 (d, C-7), 63.15, 73.10 (s, C-1, C-10), 82.19 (d, C-6), 110.67 (t, C-14), 148.28 (s, C-4), 178.65 (s, C-13); HREIMS *m/z* 248.1414 (calcd for $C_{15}H_{20}O_3$ 248.1413).

The second peak (t_R 5.2 min) gave **22** (58.8 mg, 41%) as colorless needles (EtOAc): mp 63−64 °C; $[\alpha]^{20}_D$ −21.2° (*c* 0.53, $CHCl_3$); IR ($CHCl_3$) ν_{max} 1774 cm^{-1}; ^1H NMR δ 1.23 (3H, d, *J* = 6.7 Hz, H-12), 1.36 (3H, s, H-15), 2.29 (1H, dq, *J* = 12.6, 6.7 Hz, H-11), 2.61 (1H, d, *J* = 11.3 Hz, H-5), 3.74 (1H, dd, *J* = 11.3, 9.8 Hz, H-6), 5.15 (2H, br s, H-14); ^{13}C NMR δ 12.42 (q, C-12), 20.49 (q, C-15), 25.00 (t), 30.04 (t), 30.44 (t), 37.42 (t), 42.25 (d, C-11), 53.96 (d, C-5), 55.52 (d, C-7), 62.82, 72.51 (s, C-1, C-10), 81.28 (d, C-6), 112.06 (t, C-14), 147.04 (s, C-4), 177.74 (s, C-13); *anal*. C 72.68, H 8.08, calcd for $C_{15}H_{20}O_3$, C 72.55, H 8.12.

Phenylselenenylation of a mixture (1:1.6:4) of 16, 17, and 1. Formation of 11α-(Phenylseleno)guaia-4(14),10(15)-dieno-13,6α-lactone (23), 11α-(Phenylseleno)guaia-4(14),9-dieno-13,6α-lactone (24), 11β-(Phenylseleno)guaia-4(14),9-dieno-13,6α-lactone (25), 11β-(Phenylseleno)guaia-1(10),4(14)-dieno-13,6α-lactone (26), and 11β-(Phenylseleno)guaia-4(14),10(15)-dieno-13,6α-lactone (27). A solution of the mixture of **16, 17**, and **1** (400 mg, 1.72 mmol) in THF (6 mL) was slowly added to a cooled (−76 °C) solution of lithium diisopropylamide [prepared from diisopropylamine (746 μL, 5.31 mmol), 1.6 M BuLi in hexane (3.3 μL, 5.31 mmol)] in THF (6 mL). After 1 h, a solution of diphenyl diselenide (1.66 g, 5.31 mmol) in THF (5 mL) and HMPA (926 μL, 5.31 mmol) was added at −76 °C. The reaction mixture was stirred at −76 °C for 30 min and then warmed to −40 °C over period of 30 min, and stirring was continued for an additional 1.5 h. The reaction was quenched by addition of 0.2 M aqueous solution of HCl (40 mL) at −10 °C. The mixture was extracted with EtOAc (3 × 20 mL). The combined extracts were worked up to give a crude oily product (2.3 g), which was passed through a short column of silica gel. The eluent was then separated by HPLC [B, EtOAc−hexane (5:95), 3.0 mL/min].

The first peak (t_R 2.8 min) gave **23** (47.7 mg, 7%) as a pale yellow oil: IR ($CHCl_3$) ν_{max} 1770, 1644 cm^{-1}; ^1H NMR δ 1.42 (3H, s, H-12), 3.91 (1H, dd, *J* = 9.6, 9.6, H-6), 4.74 (1H, br s, H-15a), 4.84 (1H, br s, H-15b), 5.00 (1H, br s, H-14a), 5.09 (1H, br s, H-14b), 7.27−7.42 (3H, m, Ph), 7.67−7.73 (2H, m, Ph).

The second peak (t_R 3.6 min) gave a mixture (1:6) of **24** and **25** (168.2 mg, 25%). Repeated purification of this mixture by HPLC employing the same conditions gave spectroscopically pure **25** as colorless crystals: mp 104−106 °C; $[\alpha]^{20}_D$ +95.4° (*c* 0.31, $CHCl_3$); IR ($CHCl_3$) ν_{max} 1758, 1662 cm^{-1}; ^1H NMR (500 MHz) δ 1.53 (3H, s, H-12), 1.82 (3H, br s, H-15), 1.96 (1H, ddd, *J* = 11.5, 10.1, 3.2 Hz, H-7), 4.17 (1H, dd, *J* = 10.1, 10.1 Hz, H-6), 4.99 (1H, br s, H-14a), 5.18 (1H, br s, H-14b), 5.57 (1H, ddd, *J* = 8.1, 2.7, 1.5 Hz, H-9), 7.27−7.45 (3H, m, Ph), 7.58−7.68 (2H, m, Ph); ^{13}C NMR δ 22.10 (q, C-12), 26.82 (t, C-8), 27.84 (q, C-15), 29.14 (t), 29.80 (t), 47.58 (d, C-5), 51.28 (s, C-11), 51.56 (d, C-1), 52.69 (d, C-7), 81.42 (d, C-6), 110.56 (t, C-14), 121.25 (d, C-9), 124.55 (s, Ph), 128.94 (d, *m*-Ph), 129.72 (d, *p*-Ph), 138.33 (d, *o*-Ph), 138.44 (s, C-10), 150.28 (s, C-4), 175.76 (s, C-13). ^1H NMR of **24** (scanned on the mixture of **24** and **25**) δ 1.41 (3H, s, H-12), 1.77 (3H, br s, H-15), 3.99 (1H, dd, *J* = 9.6, 9.6 Hz, H-6), 4.95 (1H, br s, H-14a), 5.08 (1H, br s, H-14b), 5.45−5.55 (1H, m, H-9), 7.27−7.45 (3H, m, Ph), 7.68−7.72 (2H, m, Ph).

The third peak (t_R 4.4 min) gave **26** (78.5 mg, 12%) as a pale yellow oil: $[\alpha]^{20}_D$ +69.9° (*c* 0.39, $CHCl_3$); IR ($CHCl_3$) ν_{max} 1768 cm^{-1}; ^1H NMR δ 1.53 (3H, s, H-12), 1.77 (3H, br s, H-15), 2.06 (1H, ddd, *J* = 10.3, 10.3, 2.9 Hz, H-7), 3.20 (1H, br d, *J* = 10.3 Hz, H-5), 4.08 (1H, dd, *J* = 10.3, 10.3 Hz, H-6), 5.09 (1H,

br s, H-14a), 5.14 (1H, br s, H-14b), 7.28−7.44 (3H, m, Ph), 7.58−7.64 (2H, m, Ph); ^{13}C NMR δ 21.84 (q, C-12), 23.04 (q, C-15), 25.46 (t), 31.57 (t), 32.74 (t), 34.71(t), 50.77 (s, C-11), 51.99 (d, C-5), 60.51 (d, C-7), 79.48 (d, C-6), 110.61 (t, C-14), 124.21 (s, Ph), 128.86 (d, *m*-Ph), 129.58 (d, *p*-Ph), 131.79 (s, C-1), 135.91 (s, C-10), 138.29 (d, *o*-Ph), 149.68 (s, C-4), 175.43 (s, C-13); HREIMS *m*/*z* 388.0950 (calcd for $C_{21}H_{24}O_2Se$ 388.0942).

The fourth peak (t_R 6.4 min) gave a mixture (1:4.3) of **17** and **1** (23.22 mg, 6%).

The fifth peak (t_R 7.2 min) gave **27** (269.6 mg, 41%) as colorless prisms (EtOAc): mp 127−128 °C; $[\alpha]^{20}_D$ +19.3° (*c* 0.92, $CHCl_3$); IR ($CHCl_3$) ν_{max} 1754, 1644 cm^{-1}; ^{1}H NMR δ 1.55 (3H, s, H-12), 2.14 (1H, dd, *J* = 9.5, 3.5 Hz, H-5), 4.08 (1H, dd, *J* = 9.5, 9.5 Hz, H-6), 4.81 (1H, s, H-15a), 4.92 (1H, s, H-15b), 5.05 (1H, d, *J* = 2.1 Hz, H-14a), 5.15 (1H, d, *J* = 1.8 Hz, H-14b), 7.28−7.44 (3H, m, Ph), 7.58−7.64 (2H, m, Ph); ^{13}C NMR δ 22.28 (q, C-12), 29.76 (t), 29.91 (t), 32.14 (t), 37.01 (t), 47.10 (d, C-1), 51.40 (s, C-11), 51.78 (d, C-7), 54.60 (d, C-5), 82.49 (d, C-6), 109.73 (t, C-15), 112.13 (t, C-14), 124.75 (s, Ph), 128.92 (d, *m*-Ph), 129.72 (d, *p*-Ph), 138.35 (d, *o*-Ph), 149.85 (s, C-10), 151.08 (s, C-4), 175.82 (s, C-13); HREIMS *m*/*z* 388.0949 (calcd for $C_{21}H_{24}O_2Se$ 388.0942).

Guaia-4(14),7(11),10(15)-trieno-13,6α-lactone (28). A solution of **23** (4.2 mg, 0.01 mmol) in THF (200 μL) containing AcOH (2.0 μL, 0.03 mmol) was treated at 0 °C with 31% H_2O_2 (6.0 μL, 0.06 mmol) for 1 h. The reaction mixture was poured into a saturated aqueous solution of $NaHCO_3$ (5 mL) and extracted with EtOAc (3 × 4 mL). The combined extracts were worked up to give a crude oily product (3.5 mg), which was purified by HPLC [A, EtOAc−hexane (2:8), 2.4 min] to give **28** (2.2 mg, 87%) as colorless plates (EtOAc): mp 82−84 °C; $[\alpha]^{20}_D$ +28.1° (*c* 0.17, $CHCl_3$); IR ($CHCl_3$) ν_{max} 1744, 1670 cm^{-1}; ^{1}H NMR δ 1.80 (H, br s, H-12), 2.49 (1H, m, H-5), 2.88 (1H, m, H-1), 4.63 (1H, br d, *J* = 12.1 Hz, H-6), 4.94 (1H, d, *J* = 1.7 Hz, H-15a), 4.96 (1H, br s, H-15b), 5.11 (1H, br s, H-14a), 5.19 (1H, br s, H-14b); ^{13}C NMR δ 8.58 (q, C-12), 28.91 (t), 29.74 (t), 30.41 (t), 30.68 (t), 48.86 (d, C-1), 51.57 (d, C-5), 80.63 (d, C-6), 111.21 (s), 112.21 (t, C-14), 113.12 (t, C-15), 122.97 (s), 149.28 (s, 2C), 162.73 (s, C-13); HREIMS *m*/*z* 230.1308 (calcd for $C_{15}H_{18}O_2$ 230.1307).

Eremanthin (3) and Guaia-4(14),7(11),9-trieno-13,6α-lactone (29). The oxidative syn elimination of a mixture (1:6) of **24** and **25** (152.8 mg, 0.39 mmol) by the method mentioned above gave a mixture of eremanthin (**3**) and the endo-unsaturated γ-lactone (**29**), which were separated by HPLC [A, EtOAc−hexane (5:95), 3.0 mL/min]. The first peak (t_R 2.8 min) gave **3** (69.3 mg, 76%) as colorless needles (EtOAc): mp 63 °C; $[\alpha]^{20}_D$ −106.6° (*c* 0.55, $CHCl_3$); IR ($CHCl_3$) ν_{max} 1762, 1660 cm^{-1}; ^{1}H NMR (500 MHz) δ 1.54 (1H, m, H-2), 1.84 (3H, br s, H-15), 2.01 (1H, m, H-8), 2.17 (1H, m, H-2), 2.37 (1H, m, H-3), 2.50−2.73 (5H, m, H-1, H-3, H-5, H-7, H-8), 3.94 (1H, dd, *J* = 10.3, 9.4 Hz, H-6), 5.04 (1H, br s, H-14a), 5.21 (1H, br s, H-14b), 5.47 (1H, d, *J* = 3.2 Hz, H-12a), 5.53 (1H, br d, *J* = 7.7 Hz, H-9), 6.20 (1H, d, *J* = 3.4 Hz, H-12b); ^{13}C NMR (125 MHz) δ 27.95 (q, C-15), 29.10 (t, C-8), 29.52 (t, C-3), 30.51 (t, C-2), 45.27 (d, C-7), 47.03 (d, C-1), 52.49 (d, C-5), 83.21 (d, C-6), 110.92 (t, C-14), 119.39 (t, C-12), 120.68 (d, C-9), 138.09 (s, C-10), 139.99 (s, C-11), 149.92 (s, C-4), 170.00 (s, C-13); *anal*. C 77.94%, H 8.07%, calcd for $C_{15}H_{18}O_2$, C 78.23%, H 7.88%.

The second peak (t_R 4.6 min) gave **29** (11.9 mg, 13%) as a colorless oil: $[\alpha]^{20}_D$ +91.2° (*c* 0.07, $CHCl_3$); IR ($CHCl_3$) ν_{max} 1744, 1674 cm^{-1}; ^{1}H NMR (500 MHz) δ 1.78 (3H, br s, H-15), 1.81 (3H, dd, *J* = 3.2, 1.5 Hz, H-12), 1.94 (2H, m, H-2), 2.39 (1H, dd, *J* = 9.2, 7.7 Hz, H-5), 2.51 (1H, m, H-3), 2.89 (1H, dd, *J* = 13.2, 7.7 Hz, H-1), 3.13 (1H, dd, *J* = 18.6, 6.7 Hz, H-8), 3.29 (1H, d, *J* = 18.6 Hz, H-8), 4.67 (1H, br d, *J* = 9.2 Hz, H-6), 5.05 (1H, dd, *J* = 4.4, 2.2 Hz, H-14a), 5.29 (1H, dd, *J* = 4.4, 2.3 Hz, H-14b), 5.49 (1H, m, H-9); ^{13}C NMR (125 MHz) δ 8.41 (q, C-15), 22.94 (q, C-12), 25.91 (t, C-8), 27.26 (t, C-2), 30.82 (t, C-3), 43.74 (d, C-1), 51.17 (d, C-5), 82.85 (d, C-6), 108.95 (t, C-14), 118.23 (d, C-9), 139.63 (s, C-10), 151.12 (s, 2C), 160.57 (s, C-7), 174.58 (s, C-13); HREIMS *m*/*z* 230.1320 (calcd for $C_{15}H_{18}O_2$ 230.1307).

Guaia-1(10),4(14),11(12)-trieno-13,6α-lactone (30). The oxidative syn elimination of **26** by the method mentioned above gave **30** (61%) as a colorless oil: IR ($CHCl_3$) ν_{max} 1768 cm^{-1}; ^{1}H NMR δ 1.76 (3H, br s, H-15), 3.33 (1H, br d, *J* = 10.2 Hz, H-5), 3.64 (1H, dd, *J* = 10.2, 10.2 Hz, H-6), 5.10 (1H, br s, H-14a), 5.17 (1H, br s, H-14b), 5.38 (1H, d, *J* = 3.1 Hz, H-12a), 6.11 (1H, *J* = 3.3 Hz, H-12b); ^{13}C NMR δ 23.34 (q, C-15), 25.87 (t, C-8), 31.78 (t), 32.82 (t), 34.45 (t, C-9), 52.40 (d, C-5), 53.14 (d, C-7), 81.97 (d, C-6), 110.61 (t, C-14), 117.52 (t, C-12), 131.43 (s), 136.00 (s), 140.42 (s, C-11), 149.52 (s, C-4), 169.81 (s, C-13); HREIMS *m*/*z* 230.1307 (calcd for $C_{15}H_{18}O_2$ 230.1307).

Dehydrocostus Lactone (2). The oxidative syn elimination of **27** by the method mentioned above gave dehydrocostus lactone **2** (99%) as colorless needles (EtOAc): mp 49 °C; $[\alpha]^{20}_D$ −14.8° (*c* 0.42, $CHCl_3$); IR ($CHCl_3$) ν_{max} 1762, 1644 cm^{-1}; ^{1}H NMR (500 MHz) δ 1.42 (1H, dddd, *J* = 13.2, 11.5, 10.2, 5.6 Hz, H-8), 1.87 (1H, ddd, *J* = 13.2, 4.7, 2.7 Hz, H-2), 1.95 (1H, ddd, *J* = 13.2, 7.6, 1.7 Hz, H-2), 2.16 (1H, dddd, *J* = 12.2, 10.4, 5.6, 5.6 Hz, H-9), 2.24 (1H, dddd, *J* = 13.2, 8.2, 5.8, 4.4 Hz, H-8), 2.87 (1H, dd, *J* = 9.3, 3.0 Hz, H-5), 2.88−2.95 (2H, m, H-1, H-7), 3.97 (1H, dd, *J* = 9.3, 9.3 Hz, H-6), 4.82 (1H, s, H-15a), 4.90 (1H, s, H-15b), 5.07 (1H, br d, *J* = 2.0 Hz, H-14a), 5.27 (1H, br d, *J* = 2.0 Hz, H-14b), 5.49 (1H, d, *J* = 3.2 Hz, H-12a), 6.22 (1H, d, *J* = 3.3 Hz, H-12b); ^{13}C NMR (125 MHz) δ 30.28 (t, C-2), 30.91 (t, C-8), 32.57 (t, C-3), 36.24 (t, C-9), 45.11 (d, C-7), 47.60 (d, C-1), 52.01 (d, C-5), 85.22 (d, C-6), 109.59 (t, C-14), 112.60 (t, C-15), 120.16 (t, C-12), 139.73 (s, C-11), 149.20 (s, C-10), 151.21 (s, C-4), 170.24 (s, C-13); *anal*. C 77.67%, H 7.97%, calcd for $C_{15}H_{18}O_2$, C 78.23%, H 7.88%.

Selective Epoxidation of 17 in a Mixture (1:4.3) of 17 and Mokko Lactone (1) To Give 9α,10α-Epoxyguai-4(14)-eno-13,6α-lactone (31). A solution of a mixture (1:4.3) of **17** and **1** (42 mg, 0.064 mmol) in $CHCl_3$ (0.8 mL) was stirred with 78% *m*-CPBA (2.94 mg, 0.013 mmol) for 1 h at 0 °C. The reaction mixture was worked up to give a colorless oil (15 mg), which was purified by the combination of column chromatography [silica gel, 2 g; 1.2-cm i.d., EtOAc−hexane (1:9 and 2:8)] and HPLC [A, EtOAc−hexane (5:95), 3.0 mL/min]. The peak (t_R 6.8 min) gave Mokko Lactone (**1**) (4.0 mg, 48%) on evaporation of the solvent. Slow elution gave **31** (2.1 mg, 13%) as colorless plates (EtOAc): mp 114−116 °C; $[\alpha]^{20}_D$ −12.0° (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) ν_{max} 1766, 1660 cm^{-1}; ^{1}H NMR (500 MHz) δ 1.23 (3H, d, *J* = 6.8 Hz, H-12), 1.38 (3H, s, H-15), 1.66 (1H, m, H-3), 1.84 (1H, dd, *J* = 15.1, 12.0 Hz, H-8*ax*), 2.00 (1H, dddd, *J* = 12.3, 12.0, 10.1, 2.7 Hz, H-7), 2.06 (1H, m, H-2), 2.27 (1H, dq, *J* = 12.3, 6.8 Hz, H-11), 2.40 (1H, ddddd, *J* = 17.6, 9.3, 9.3, 2.2, 2.2 Hz, H-3), 2.49 (1H, ddd, *J* = 15.1, 5.4, 2.7 Hz, H-8*eq*), 2.52−2.62 (3H, m, H-1, H-2, H-5), 3.03 (1H, d, *J* = 5.4 Hz, H-9), 3.65 (1H, dd, *J* = 10.1, 10.1 Hz, H-6), 4.95 (1H, br s, $W_{h/2}$ = 5.0 Hz, H-14a), 5.10 (1H, br s, $W_{h/2}$ = 5.0 Hz, H-14b); HREIMS *m*/*z* 248.1409 (calcd for $C_{15}H_{20}O_3$ 248.1416).

9α,10α-Epoxy-11β-(phenylseleno)guaia-4(14)-eno-13,6α-lactone (32). Phenylselenenylation of **31** gave **32** and unreacted **31**, which were separated by flash chromatography. The first elution gave **32** (44%) as colorless needles (EtOAc): mp 152−154 °C; IR ($CHCl_3$) ν_{max} 1760, 1660 cm^{-1}; ^{1}H NMR δ 1.38 (3H, s, H-15), 1.53 (3H, s, H-12), 3.10 (1H, d, *J* = 5.5 Hz, H-9), 3.82 (1H, dd, *J* = 9.8, 9.8 Hz, H-6), 4.94 (1H, br s, $W_{h/2}$ = 5.0 Hz, H-14a), 5.09 (1H, br s, $W_{h/2}$ = 5.0 Hz, H-14b), 7.26−7.47 (3H, m, Ph), 7.55−7.65 (2H, m, Ph).

Further flash chromatography gave recovered **31** (36%).

9α, 10α-Epoxyguaia-4(14),11(12)-dieno-13,6α-lactone (33). The oxidative syn elimination of **32** gave **33** (95%) as colorless needles (EtOAc): mp 136−138 °C; $[\alpha]^{20}_D$ −36.6° (*c* 0.40, $CHCl_3$); IR ($CHCl_3$) ν_{max} 1770, 1664 cm^{-1}; ^{1}H NMR (500 MHz) δ 1.39 (3H, s, H-15), 1.66 (1H, dddd, *J* = 12.9, 12.9, 10.7, 8.8 Hz, H-2*ax*), 1.91 (1H, dd, *J* = 15.4, 12.0 Hz, H-8*ax*), 2.07 (1H, m, H-2*eq*), 2.40 (1H, ddddd, *J* = 17.6, 8.8, 8.8, 2.0, 2.0 Hz, H-3), 2.55 (1H, m, H-3), 2.63 (1H, dd, *J* = 12.9, 6.2 Hz, H-1), 2.68 (1H, dd, *J* = 10.7, 6.2 Hz, H-5), 2.63 (1H, ddd, *J* = 15.4, 9.3, 4.9 Hz, H-8*eq*), 2.90 (1H, dddd, *J* = 12.0, 9.5, 3.4, 3.4 Hz, H-7), 3.09 (1H, d, *J* = 4.9 Hz, H-9), 3.65 (1H, dd, *J* = 10.7, 9.5 Hz, H-6), 4.99 (1H, br s, $W_{h/2}$ = 4.0 Hz, H-14a), 5.12 (1H, br s, $W_{h/2}$ = 4.0 Hz, H-14b), 5.48 (1H, d, *J* = 3.4 Hz, H-12a), 6.19 (1H, d, *J* = 3.4 Hz, H-12b); ^{13}C NMR (125 MHz)

δ 26.23 (q, C-15), 27.63 (t), 28.29 (t), 28.37 (t), 40.90 (d, C-7), 46.61 (d), 52.07 (d), 61.64 (d, C-9), 63.40 (s, C-10), 81.58 (d, C-6), 111.16 (t, C-14), 119.11 (t, C-12), 139.59 (s, C-11), 148.50 (s, C-4), 169.66 (s, C-13); HREIMS *m/z* 246.1257 (calcd for $C_{15}H_{18}O_3$ 246.1256).

Phenylselenenylation of 21. Formation of 1β,10β-Epoxy-11α-(phenylseleno)guaia-4(14)-eno-13,6α-lactone (34) and 1β,10β-Epoxy-11β-(phenylseleno)guaia-4(14)-eno-13,6α-lactone (35). Phenylselenenylation of **21** gave **34** and **35**, which were separated by flash chromatography. The first elution gave **34** (7%) as a pale yellow oil: ^{1}H NMR δ 1.33 (3H, s, H-15), 1.40 (3H, s, H-12), 2.49 (1H, br d, J = 9.8 Hz, H-5), 4.10 (1H, dd, J = 9.8, 9.8 Hz, H-6), 5.08 (1H, d, J = 2.1 Hz, H-14a), 5.33 (1H, d, J = 2.2 Hz, H-14b), 7.27−7.45 (3H, m, Ph), 7.60−7.70 (2H, m, Ph).

The second elution gave **35** (78%) as a pale yellow oil: IR ($CHCl_3$) ν_{max} 1764, 1662 cm^{-1}; ^{1}H NMR δ 1.37 (3H, s, H-15), 1.49 (3H, s, H-12), 4.35 (1H, dd, J = 10.1, 9.2 Hz, H-6), 5.12 (1H, d, J = 1.9 Hz, H-14a), 5.41 (1H, d, J = 2.1 Hz, H-14b), 7.27−7.44 (3H, m, Ph), 7.58−7.65 (2H, m, Ph); ^{13}C NMR δ 21.06 (t), 21.84 (q, C-15), 22.86 (q, C-12), 31.09 (t), 32.41 (t), 34.25 (t), 49.06 (d, C-5), 50.01 (s, C-11), 57.37 (d, C-7), 62.84 (s), 72.88 (s), 80.22 (d, C-6), 110.71 (t, C-14), 124.33 (s, Ph), 128.88 (d, *m*-Ph), 129.63 (d, *p*-Ph), 138.29 (d, *o*-Ph), 148.15 (s, C-4), 175.96 (s, C-13).

1β,10β-Epoxyguaia-4(14),7(11)-dieno-13,6α-lactone (36). The oxidative syn elimination of **34** gave spectroscopically pure **36** (86%) as a colorless oil: IR ($CHCl_3$) ν_{max} 1764, 1646 cm^{-1}; ^{1}H NMR δ 1.39 (3H, s, H-15), 1.80 (3H, s, H-12), 4.74 (1H, d, J = 10.3 Hz, H-6), 5.19 (1H, br s, $W_{h/2}$ = 4.0 Hz, H-14a), 5.43 (1H, br s, H-14b); HREIMS *m/z* 246.1256 (calcd for $C_{15}H_{18}O_3$ 246.1256).

1β,10β-Epoxyguaia-4(14),11(12)-dieno-13,6α-lactone (37). The oxidative syn elimination of **35** gave **37** (85%) as colorless plates (EtOAc): mp 125−126 °C; $[\alpha]^{20}_D$ +34.6° (*c* 0.10, $CHCl_3$); IR ($CHCl_3$) ν_{max} 1766, 1660 cm^{-1}; ^{1}H NMR (500 MHz) δ 1.38 (3H, s, H-15), 1.49 (1H, dddd, J = 13.9, 12.9, 11.2, 2.7 Hz, H-8), 1.68 (1H, ddd, J = 13.9, 8.8, 3.7 Hz, H-2), 1.86 (1H, dddd, J = 13.9, 4.7, 3.3, 1.7 Hz, H-8), 2.05 (1H, ddd, J = 15.6, 12.9, 3.3 Hz, H-9), 2.15 (1H, ddd, J = 13.9, 9.8, 9.8 Hz, H-2), 2.24 (1H, ddd, J = 15.6, 4.7, 2.7 Hz, H-9), 2.30 (1H, ddd, J = 11.2, 10.3, 1.7 Hz, H-7), 2.48 (1H, m, H-3), 2.61 (1H, m, H-3), 2.94 (1H, br dd, J = 10.3, 2.3 Hz, H-5), 3.98 (1H, dd, J = 10.3, 10.3 Hz, H-6), 5.15 (1H, dd, J = 4.6, 2.0 Hz, H-14a), 5.44 (1H, d, J = 2.9 Hz, H-12a), 5.47 (1H, dd, J = 4.4, 2.2 Hz, H-14b), 6.18 (1H, d, J = 3.4 Hz, H-12b); ^{13}C NMR (125 MHz) δ 21.70 (t, C-8), 23.01 (q, C-15), 31.02 (t, C-3), 32.44 (t, C-2), 34.04 (t, C-9), 49.39 (d, C-5), 49.70 (d, C-7), 63.25 (s, C-10), 73.10 (s, C-1), 82.38 (d, C-6), 110.90 (t, C-14), 118.65 (t, C-12), 138.89 (s, C-11), 147.93 (s, C-4), 170.11 (s, C-13); *anal.* C 72.99%, H 7.43%, calcd for $C_{15}H_{18}O_3$, C 73.14%, H 7.37%.

1α,10α-Epoxy-11β-(Phenylseleno)guaia-4(14)-eno-13,6α-lactone (38). Phenylselenenylation of **22** gave **38** and recovered **22**, which were separated by flash chromatography. The first elution gave **38** (60%) as colorless needles (EtOAc): mp 218−220 °C; $[\alpha]^{20}_D$ −33.0° (*c* 0.09, $CHCl_3$); IR ($CHCl_3$) ν_{max} 1772, 1660 cm^{-1}; ^{1}H NMR δ 1.39 (3H, s, H-15), 1.55 (3H, s, H-12), 2.55 (1H, d, J = 11.2, Hz, H-5),4.03 (1H, dd, J = 11.2, 9.5 Hz, H-6), 5.15 (2H, br s, H-14), 7.27−7.46 (3H, m, Ph), 7.60−7.68 (2H, m, Ph); ^{13}C NMR δ 20.41 (q, C-15), 21.97 (q, C-12), 22.99 (t), 29.89 (t), 30.29 (t), 37.28 (t), 50.94 (s, C-11), 54.06 (d, C-5), 59.55 (d, C-7), 62.76 (s), 72.33 (s), 79.14 (d, C-6), 112.16 (t, C-14), 124.18 (s, Ph), 129.03 (d, *m*-Ph), 129.79 (d, *p*-Ph), 138.16 (d, *o*-Ph), 146.92 (s, C-4), 175.13 (s, C-13); HREIMS *m/z* 404.0918 (calcd for $C_{21}H_{24}O_3Se$ 404.0891). The second elution gave recovered **22** (14%).

1α,10α-Epoxyguaia-4(14),11(12)-dieno-13,6α-lactone (39). The oxidative syn elimination of **38** gave **39** (84%) as colorless needles (EtOAc): mp 130−131 °C; $[\alpha]^{20}_D$ −54.7° (*c* 0.17, $CHCl_3$); IR ($CHCl_3$) ν_{max} 1774, 1662 cm^{-1}; ^{1}H NMR (500 MHz) δ 1.36 (3H, s, H-15), 1.46 (1H, dddd, J = 13.7, 12.9, 10.7, 1.2 Hz, H-8), 1.57 (1H, m, H-9), 1.74 (1H, dddd, J = 14.7, 9.3, 5.4, 1.0 Hz, H-3), 2.16 (1H, dddd, J = 13.7, 6.7, 3.2, 1.5 Hz, H-8), 2.21 (1H, ddd, J = 14.7, 10.1, 8.1 Hz, H-2), 2.34 (1H, ddd, J = 14.3, 6.7, 1.2 Hz, H-9), 2.52 (1H, m, H-3), 2.58−2.69 (2H, m, H-2, H-7), 2.73 (1H, br d, J = 11.0 Hz, H-5), 3.75 (1H, dd, J = 11.0, 10.0 Hz, H-6), 5.18 (2H, d, J = 1.2 Hz, H-14), 5.44 (1 H, d, J = 3.0 Hz, H-12a), 6.16 (1H, d, J = 3.5 Hz, H-12b); ^{13}C NMR (125 MHz) δ 20.69 (q, C-15), 23.52 (t, C-8), 30.02 (t, C-2), 30.45 (t, C-3), 36.89 (t, C-9), 51.90 (d, C-7), 54.19 (d, C-5), 67.72 (s, C-10), 72.61 (s, C-1), 81.35 (d, C-6), 112.18 (t, C-14), 118.48 (t, C-12), 139.72 (s, C-11), 146.79 (s, C-4), 169.32 (s, C-13); *anal.* C 72.69%, H 7.33%, calcd for $C_{15}H_{18}O_3$, C 73.14%, H 7.37%.

Materials and Methods for Bioassays

Cells. A mouse H-2^d-specific CD8$^+$ CTL clone, OE4, and a human lung adenocarcinoma clone, A549, were maintained in RPMI 1640 medium supplemented with 10% (v/v) fetal calf serum, 5% (v/v) rat-conditioned medium (culture supernatant of rat splenocytes stimulated with 5 mg/mL of concanavalin A for 24 h), 50 mM 2-mercaptoethanol, 50 mg/mL of kanamycin, and 8 mg/mL of tylosin tartrate. OE4 was stimulated with mitomycin C-treated splenocytes from BALB/c mice (female, 6−8 weeks old, Charles River Co., Ltd., Yokohama, Japan) every other week and used for experiments at least 5 days after the stimulation. P815 mastocytoma was maintained in the medium described above, but without rat-conditioned medium.

Antibodies and Cytokine. C167 (mouse anti human ICAM-1 antibody) was purchased from Leinco Technologies Inc., Ballwin, MO, and sheep peroxidase-linked anti-mouse Ig antibody was from Amersham International, Buckinghamshire, U.K. Recombinant human IL-1β was a commercial product of genzyme diagnostics, Cambridge, MA.

Cytotoxicity Assay. The target cell (TC) P815(H-2^d) was incubated with [^{3}H]thymidine (1 mci/mL) for 18 h and washed three times with the medium before use. OE4 (1 × 10^4 cells/well in 100 mL) was incubated with or without test compounds for 2 h in U-bottom microtiter plates and mixed with 100 mL of TC P815 (1 × 10^4 cells/mL). The plates were centrifuged (300*g*) for 3 min and incubated for 4 h. To assess DNA degradation, 10 mL of 2% (v/v) Triton X-100 was added to each well at the end of the incubation, and the cells were solubilized by pipetting and centrifuged (600*g*, 5 min). One hundred microliters of supernatant was removed and measured for radioactivity. The percentage of specific lysis was calculated by using the following formula as described in a previous paper:[20]

$$\% \text{ of specific lysis} = (\text{experimental release} - \text{spontaneous release})/(\text{maximum release} - \text{spontaneous release}) \times 100$$

Assay of ICAM-1 Expression. After 1 h of A549 cells with or without test compounds in a microtiter plate at 3 × 10^4 cells/well in 150 mL, 50 mL of IL-1β was added to the culture at a final concentration of 280 U/mL, and the cells were further incubated for 6 h. The cells were washed once with phospate-buffered saline (PBS) and fixed by 15 min of incubation with 1% paraformaldehyde−PBS for 15 min and then washed once with PBS. After blocking with 1% bovine serum albumin−PBS for 60 min at 37 °C, the cells were treated with mouse anti-human ICAM-1 antibody for 60 min at 37 °C. After being washed with 0.02% Tween-PBS for three times, the cells were treated with peroxidase-linked anti-mouse Ig antibody for 60 min at 37 °C.

The cells were washed three times with 0.02% Tween-PBS. The cells were incubated with the substrate (0.1% *o*-phenylenedianine dihydrochloride and 0.02% H_2O_2 in 0.2 M sodium citrate, pH 5.3) for 15 min in the dark and assayed for the absorbance at 415 nm by using a microplate

reader. Expression of ICAM-1 was calculated as follows.

Expression of ICAM-1 (% of control) =
(absorbance of sample − absorbance without IL-1β treatment)/
(absorbance with IL-1β treatment − absorbance without IL-1β treatment) × 100

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(28) Zn−Hg was prepared by the following procedure: Zn powder (2.5 g, 38.2 mmol) and 2 M HCl (5 mL) were stirred for 6 min, and the aqueous solution was then decanted. $HgCl_2$ (437 mg, 1.61 mmol) and 0.5 M HCl (4 mL) were added, and after being stirred for 5 min, the aqueous solution was decanted. The residue in the flask was used for the reaction immediately.

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