Chemical Transformation and Biological Activities of Ambrein, a Major Product of Ambergris from *Physeter macrocephalus* (Sperm Whale)

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Ten new derivatives (**2**−**11**) of ambrein (**1**), isolated from ambergris, were prepared by chemical transformation. Oxidation and/or cyclization were effected by reactions with selenium oxide or *p*-toluenesulfonyl chloride or with the use of shortwave UV light. The structures of **2**−**12** were elucidated by spectroscopic analysis, with the structure and relative configuration of **9** confirmed by single-crystal X-ray crystallography. The cytotoxic activities of **1**−**12** were investigated against human liver carcinoma (Hepa59T/VGH), colon adenocarcinoma (WiDr), lung carcinoma (A-549), and human breast adenocarcinoma (MCF-7) cell lines. The anti-inflammatory activities of **1**−**11**, in terms of the inhibition of human neutrophil function, were also evaluated.

Ambergris is a physiological product found either in the intestines of the sperm whale, *Physeter macrocephalus* L. (Physeteridae), or as a waste product discarded into the sea. It occurs in gray or brown waxy masses, associated with the squid on which the whales feed.[1,2] Since ancient times, ambergris has been one of the most highly valued perfumery materials and is also used in tradititional medicine to treat migraine headaches, rheumatism, and nervous diseases, and as an aphrodisiac.[3] Ambrein, a triterpene alcohol (**1**), is the major constituent of ambergris, used as a perfume additive for the tenacity or persistence of its odor,[4] and has been reported to possess anti-inflammatory, antinociceptive, and aphrodisiac activities.[5] The anti-inflammatory properties of ambrein (**1**) suggest the possible interaction of this compound and prostaglandins in the mediation of pain.[6] Oxidative degradation of ambrein (**1**), as a result of exposure of ambergris to air and sunlight, is responsible for the organoleptic properties of this compound, as it leads to formation of the odorous compound Ambrox (**13**), with wide industrial applications in perfumery.[7] In view of the medicinal potential of ambrein, we have prepared 10 new derivatives (**2**−**11**), in addition to the known compound **12**. Allylic oxidation is a useful tool for preparation of many pharmaceuticals, dyes, agricultural products, and polyester monomers.[8] Compounds **2**−**8** were produced as products of SeO_2-catalyzed allylic oxidation and benzoylation, while **9** and **10** are products of cyclization with toluenesulfonyl chloride (*p*-TsCl), and **11** and **12** are products of photooxidation by UV light (δ 254 nm). The structures of **2**−**11** were elucidated through spectroscopic data analysis, and single-crystal X-ray crystallography in the case of **9**. The cytotoxic activities of **1**−**12** were investigated against four human cancer cell lines. In addition, the anti-inflammatory activities of **1**−**11** were tested on elastase release by inhibition of human neutrophils.

Results and Discussion

The ^{1}H NMR spectrum of **1** revealed the presence of signals for seven methyls, one olefinic CH (δ_H 5.16, t, $J = 6.4$ Hz, H-13), and two exomethylene singlets at δ_H 4.75 and 4.54 (H_2-30). The ^{13}C NMR spectrum displayed resonances for one oxygenated quaternary carbon (δ_C 74.3), an olefinic CH (δ_C 124.7), a quaternary olefinic carbon (δ_C 135.9), and an exomethylene carbon (δ_C 108.9). The identity of **1** was confirmed as *E*-ambrein through mass spectrometry (elemental formula $C_{30}H_{52}O$) and from its NMR spectroscopic data, which were consistent with literature values.[9] Treatment of ambrein (**1**) with selenium dioxide in dioxane at room temperature (Scheme 1),[10,11] followed by chromatographic separation, afforded four selective oxidation products: 15α-hydroxyambrein (**2**) (7.5%), 15*β*-hydroxyambrein (**3**) (7.6%), 27-hydroxyambrein (**4**) (4.9%), and 15-oxoambrein (**5**) (0.75%).

Compounds **2** and **3** were both assigned the same molecular formula, $C_{30}H_{52}O_2$. The NMR spectroscopic data of **2** and **3** were quite similar to those of ambrein (**1**) except for the replacement of the C-15 methylene group by two oxymethines, resonating at δ_H 3.91, δ_C 75.1 in **2** and at δ_H 4.48, δ_C 67.7 in **3**. In each case, H-15 was ^3J-correlated with C-13, C-17, and C-27, confirming hydroxylation at C-15. The difference in the chemical shifts of H-15 and C-15 suggested that **2** and **3** possess different configurations at C-15. Mosher's method for the determination of the configuration of secondary alcohols was applied to **2** and **3** in order to distinguish between these two compounds.[12] In the case of **2**, the differences between the (*R*)-MTPA and (*S*)-MTPA esters ($\Delta\delta = \delta_S - \delta_R$) are summarized in Figure 1a and indicated the *S*-configuration at C-15. On the other hand, the values for ($\Delta\delta = \delta_S - \delta_R$) for **3** were indicative of the *R*-configuration at C-15 (Figure 1b).

Compound **4** exhibited a molecular formula of $C_{30}H_{52}O_2$, as determined by HRESIMS (*m*/*z* 427.3943). Its NMR data were similar to those of ambrein (**1**), but with the disappearance of the C-27 methyl group signal and its replacement by an oxymethylene signal at δ_C 66.3 in **4**. The oxymethylene protons at δ_H 4.03 (2H, s, H-27) showed HMBC correlations with the quaternary carbon at δ 139.5 (C-14), the olefinic methine carbon at δ_C 126.8 (C-13), and the methylene carbon at δ_C 30.7 (C-15), thus confirming hydroxylation at C-27. Compound **4** was assigned the structure 27-hydroxyambrein (yield 4.9%).

The FABMS of **5** revealed a $[M + Na]^+$ ion peak at *m*/*z* 465, corresponding to the molecular formula $C_{30}H_{50}O_2$. The ^{13}C NMR data of **5** displayed a low-field carbonyl signal at δ_C 201.8, in addition to 11 CH_2 signals, instead of 12 as in **1**. This carbonyl was ^3J-correlated to an olefinic proton at δ_H 6.69 (H-13), a methyl signal at δ_H 1.76 (H-27), and a methine at δ_H 2.65 (H-17), and this was accompanied by a downfield shift of H-13 ($\Delta\delta_H$ 1.53), H-16 ($\Delta\delta_H = 1.13$ ppm), and C-16 ($\Delta\delta_C = 10.7$ ppm), in comparison to

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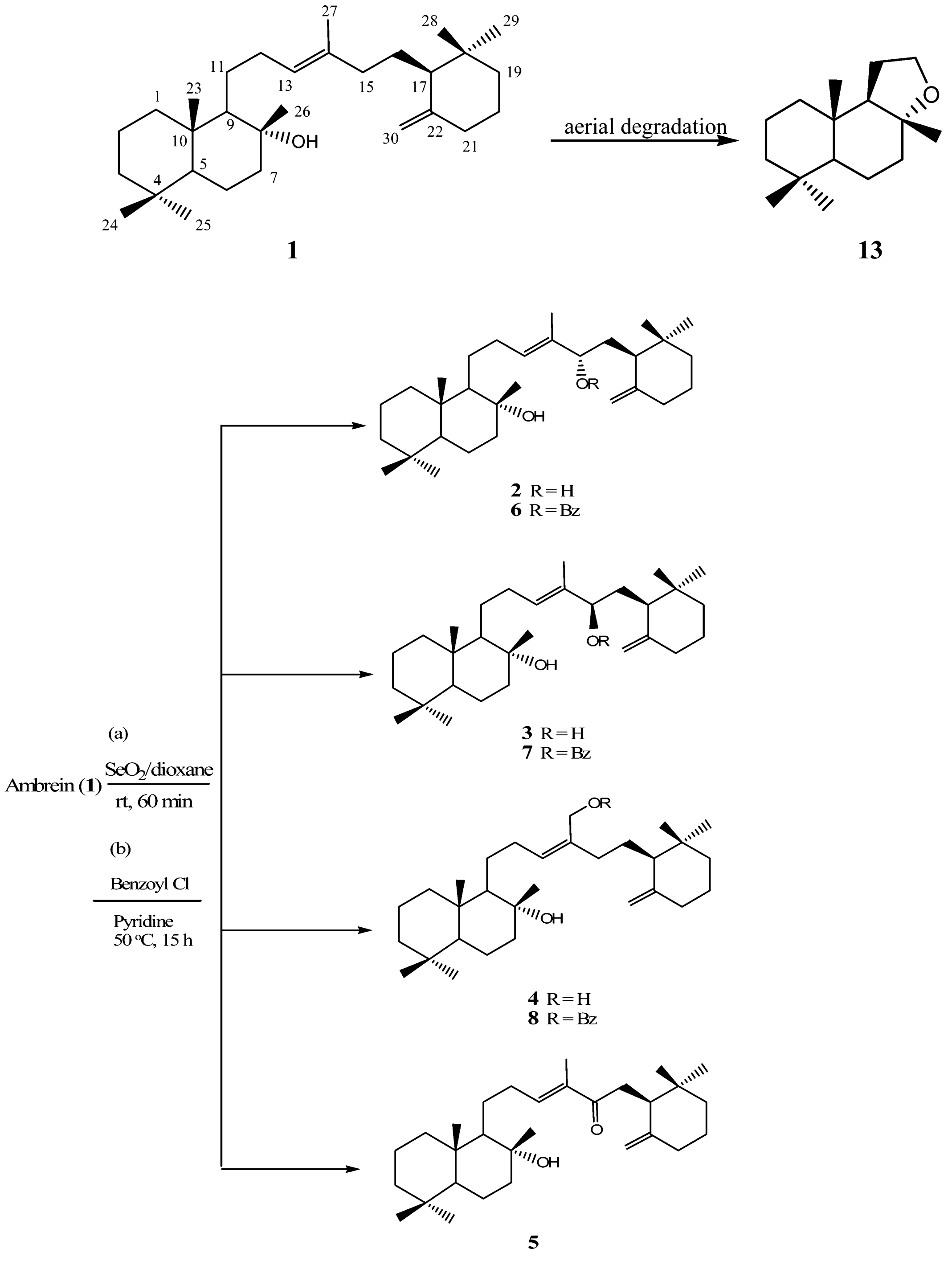
Scheme 1



1. These findings indicated the presence of a carbonyl at C-15, and consequently **5** was identified as 15-oxoambrein.

The benzoyl esters of **2**−**4** were prepared by reaction with benzoyl chloride to yield **6**−**8**, respectively, as shown in Scheme 1. The NMR spectra revealed the presence of characteristic benzoyl group signals (Experimental Section). In addition, benzoylation induced significant downfield shifts of H-15 ($\Delta\delta_H = 1.82$ ppm in **6** and $\Delta\delta_H = 0.90$ ppm in **7**) and H-27 ($\Delta\delta_H = 0.22$ ppm). The structures of these derivatives were confirmed by the HMBC correlations of the benzoyl carbonyl carbon signal in each case with H-15 (in **6** and **7**) or H-27 (in **8**).

Reaction of **3** with *p*-toluenesulfonyl chloride (TsCl)[2,13] afforded the cyclized products **9** and **10** (Scheme 2). A preferential S_N2 reaction proceeded through a nucleophilic attack that involved inversion of the orientation at C-15 in **10**.[14] The molecular formula of **9**, $C_{30}H_{50}O$, was determined from HRESIMS and NMR data. The calculated six degrees of unsaturation suggested the formation of an extra ring, relative to **1** ($C_{30}H_{52}O$). The oxymethine proton at δ_H 4.12 (dd, $J = 9.9$, 1.2 Hz, H-13) showed HMBC correlations with the methylene carbon at δ_C 15.9 (C-12) and the methyl carbon at δ_C 12.4 (C-27). The olefinic proton at δ_H 5.34 (t, $J = 6.6$ Hz, H-15) had HMBC correlations with C-16 and C-27, in addition to a NOESY correlation with H-17. These findings, together with COSY NMR spectoscopic data (Figure 2), led to the conclusion that reaction with TsCl introduced an ether linkage between C-8 and C-13, thus forming a tetrahydropyran ring that was associated with rearrangement of the double-bond position from C-13 and C-14 to C-14 and C-15. The NOESY correlation between H-13 and H-26 implied a β-orientation. A single-crystal X-ray diffraction analysis

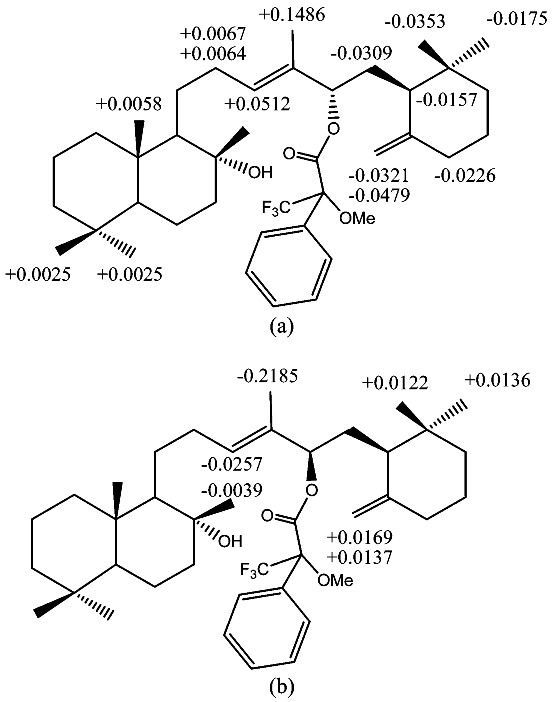


Figure 1. Δδ values obtained for the MTPA esters of (a) **2** and (b) **3**.

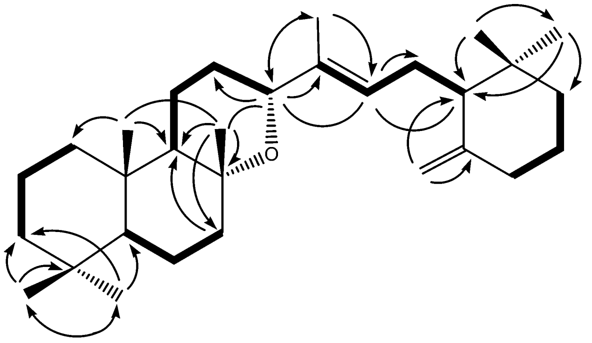
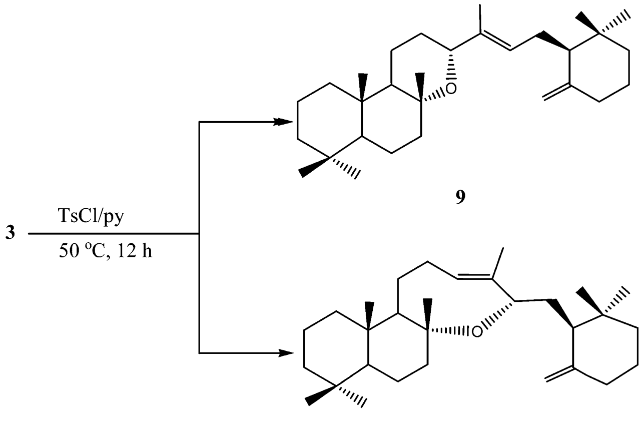


Figure 2. Selected HMBC (arrows), NOESY (curves), and COSY (bold lines) correlations of **9**.

Scheme 2



(Figure 3) was used to confirm the assignment of the structure **9**, which possesses an unusual ring C in the molecule.

Compound **10** exhibited the same molecular formula as **9**, but different NMR data, indicating a different cyclization pattern. The oxymethine proton at δ_H 3.96 (dd, $J = 9.2$, 0.9 Hz, H-15) exhibited HMBC correlations with C-13, C-16, and, notably, C-8, suggesting the presence of an oxygen bridge between C-8 and C-15. This cyclization afforded the *E*-form of an eight-membered tetrahydro-oxocine ring, which was supported by the COSY NMR spectrum (Figure 4), and proved connectivities between H-11, H-12, and H-13 and between H-15, H-16, and H-17. The strong NOESY correlation between H-15 and the C-26 methyl protons was consistent with the β-configuration of H-15.

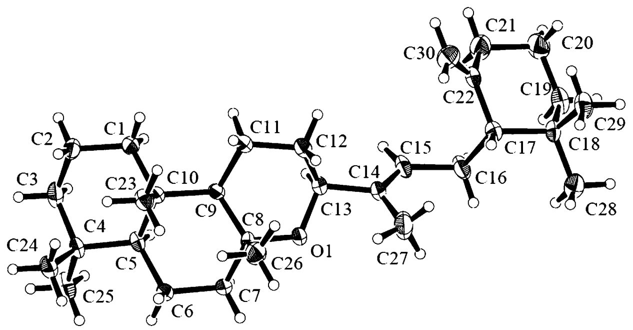


Figure 3. Perspective drawing of the X-ray structure of **9**.

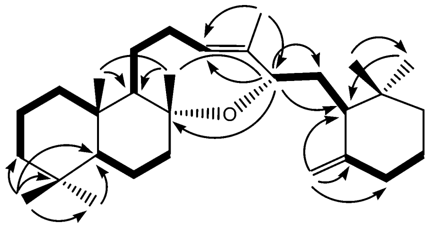
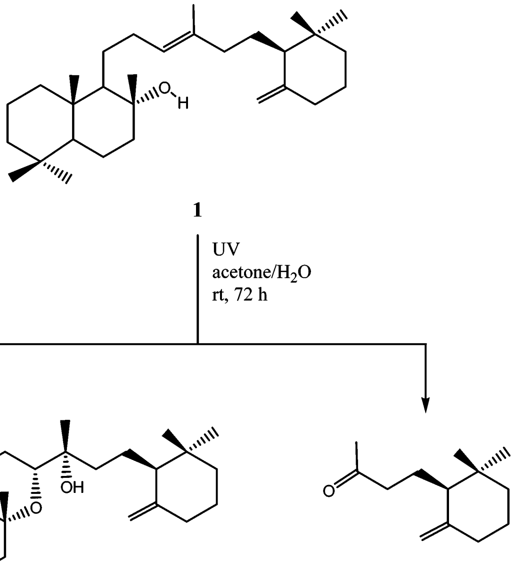


Figure 4. Key HMBC (arrows), NOESY (curves), and COSY (bold lines) correlations of **10**.

Scheme 3



Photooxidation of **1** using UV light (254 nm)[15] afforded **11** and the degradation product **12**[9] (Scheme 3). The NMR data of **11** (molecular formula $C_{30}H_{52}O_2$) indicated the absence of olefinic signals attributable to C-13 and C-14 in **1** and revealed the presence of an oxymethine at δ_H 3.38 (dd, $J = 9.9$, 2.0 Hz, H-13) and δ_C 75.2 (C-13), in addition to an oxygenated quaternary carbon at δ_C 74.9 (C-14). The oxymethine signal at δ_H 3.38 was correlated to C-8, C-15, and C-27 in the HMBC spectrum. In addition, the H-27 protons exhibited a HMBC correlation with the oxyquaternary carbon at δ_C 74.9 (C-14), confirming hydroxylation at C-14 and formation of a tetrahydropyran ring as in **9**. The strong NOESY

Table 1. Cytotoxicity of Compounds **1**−**12** against Human Tumor Cells (ED_{50}, μg/mL)[a]

compound	hepa59T/VGH[b]	WiDr[c]	A-549[d]	MCF-7[e]
2	5.7	7.8	6.4	4.0
5	6.8	10	5.3	8.5
doxorubicin	0.11	0.12	0.21	0.12

[a] Concentration that inhibits 50% of the growth of human tumor cell lines after 72 h exposure.[19] [b] Human liver carcinoma. [c] Human colon adenocarcinoma. [d] Human lung carcinoma. [e] Human breast adenocarcinoma. [f] Compounds **1**, **3**, **4**, and **6**−**12** were inactive (ED_{50} > 10 μg/mL).

Table 2. Anti-inflammatory Effect of Compounds **1**−**11** and Genistein (10 μg/mL) on Elastase Release as Tested on Human Neutrophils[a]

compound	% of control
1	4.6 ± 1.10[b]
2	132.7 ± 2.50
3	79.3 ± 5.66
4	74.4 ± 6.68
5	95.1 ± 4.63
6	115.5 ± 7.18
7	85.6 ± 7.08
8	75.6 ± 5.52
9	92.2 ± 5.62
10	82.0 ± 6.99
11	81.3 ± 4.18
genistein[c]	51.6 ± 5.89

[a] Results are presented as mean ± SEM ($n = 3$), $p < 0.05$. DMSO was used as negative control (100%). [b] Concentration necessary for 50% inhibition. [c] Positive control.

correlations between H-3 (δ_H 3.38) and H-26 (δ_H 1.26) and H-27 (δ_H 1.11) were consistent with the β-orientation of H-13 and α-orientation of the OH group at C-14. These data are in accordance with the structure assigned to **11**. Compound **12** was determined as 4-(2,2-dimethyl-6-methylenecyclohexyl)butan-2-one by spectroscopic comparison with literature data.[9] Notably, Ambrox (**13**) was not obtained under these conditions.

The in vitro cytotoxic activities of **1**−**12** were investigated against human liver carcinoma (Hepa59T/VGH), human colon adenocarcinoma (WiDr), human lung carcinoma (A-549), and human breast adenocarcinoma (MCF-7) cell lines, using the MTT assay. The results are shown in Table 1.[16] Among the compounds tested, **2** and **5** exhibited moderate cytotoxic activity against all four tumor cell lines.

The in vitro anti-inflammatory activities of **1**−**11** were also screened by observation of elastase release as tested on human neutrophils (Table 2). Genistein was used as a positive control compound. Compounds **3**, **4**, and **8** exhibited weak anti-inflammatory activities as compared to compound **1**, which showed the most potent anti-inflammatory activity at 10 μg/mL. In contrast, compounds **2** and **6** possessed inflammatory effect (over 100%) under the same conditions.

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a JASCO DIP-1000 polarimeter. IR and UV spectra were measured on Hitachi T-2001 and Hitachi U-3210 spectrophotometers, respectively. The ^{1}H, ^{13}C NMR, COSY, HMQC, HMBC, and NOESY spectra were recorded on a Bruker FT-300 or a Varian Unity INOVA FT-500 NMR spectrometer at 500 MHz for ^{1}H and 125 MHz for ^{13}C, respectively, using TMS as internal standard. Chemical shifts are given in δ (ppm) and coupling constants in Hz. Low-resolution EIMS and FABMS were recorded on a VG Quattro 5022 mass spectrometer. HRMS were recorded on a Finnigan MAT-95XL mass spectrometer. An UVGL-25 mineral light lamp (Upland, CA) operating at 254 nm was used for photoreaction. Silica gel 60 (Merck) was used for column chromatography, and precoated silica gel plates (Merck, Kieselgel 60 F-254, 1 mm) were used for preparative TLC. Sephadex LH-20 (Amersham Pharmacia Biotech AB, Uppsala, Sweden) was used for separation. *S*-(+)-α-Methoxy-α-trifluoromethylphenylacetyl chloride and *R*-(−)-α-methoxy-α-trifluoromethylphenylacetyl chloride (MTPA esters) were obtained from ACROS Organics (Geel, Belgium).

Animal Material. Ambergris, a secretion from the sperm whale *Physeter macrocephalus* L., was purchased from a local drug store in Kaohsiung, Taiwan, in June 2001. A voucher specimen (Pm-3) was deposited at the School of Pharmacy, National Taiwan University, Taipei, Taiwan.

Extraction and Isolation of Ambrein (1). The ambergris sample (20 g) was dissolved in *n*-hexane and applied to the top of a silica gel column (230−400 mesh), eluted with *n*-hexane−EtOAc (10:1), to give four fractions (F-1 to F-4). Fraction F-4 was purified on a silica gel column using *n*-hexane−EtOAc (50:1) to yield ambrein (**1**, 4.8 g, yield 24.2%).

(+)-Ambrein (1*R*,2*R*,4a*S*,8a*S*,3′*E*,1″*S*)-(+)-1-[6′-(2″,2″-Dimethyl-6″-methylenecyclohexyl)-4′-methylhex-3′-enyl]-2,5,5,8a-tetramethyldecahydronaphthalen-2-ol (1): colorless, needle-shaped crystals; mp 80−82 °C (lit. 81.5−82.5 °C); $[\alpha]_D$ +17.2 (*c* 0.2, CH_2Cl_2); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 39.8 (t, C-1), 24.9 (t, C-2), 42.1 (t, C-3), 33.2 (s, C-4), 56.2 (d, C-5), 18.5 (t, C-6), 44.6 (t, C-7), 74.3 (s, C-8), 61.5 (d, C-9), 39.2 (s, C-10), 25.6 (t, C-11), 31.5 (t, C-12), 124.7 (d, C-13), 135.9 (s, C-14), 38.3 (t, C-15), 23.8 (t, C-16), 53.7 (d, C-17), 35.0 (s, C-18), 36.4 (t, C-19), 20.6 (t, C-20), 32.6 (t, C-21), 149.4 (s, C-22), 15.5 (q, C-23), 33.3 (q, C-24), 21.6 (q, C-25), 23.8 (q, C-26), 16.4 (q, C-27), 26.3 (q, C-28), 28.5 (q, C-29), 108.9 (t, C-30); EIMS *m/z* 428 $[H]^+$; FABMS *m/z* 451 $[M + Na]^+$, 429 $[M + H]^+$, $C_{30}H_{52}O$.

Reaction of 1 with SeO_2. Selenium dioxide (885 mg, 7.90 mmol) was added to a solution of **1** (3.38 g, 7.90 mmol) in dry 1,4-dioxane (10 mL), and the reaction mixture was stirred at room temperature for 1 h. Water was then added, and the product was extracted with CH_2Cl_2. The CH_2Cl_2 layer was washed with saturated $NaHCO_3$ solution and dried over anhydrous Na_2SO_4. The solvent was evaporated and the residue (3.98 g) was chromatographed on a silica gel column (70 g) using a gradient of CH_2Cl_2−MeOH for elution to afford **1** (665 mg), a mixture M_1, **4** (164 mg, yield 4.9%), **2** (100 mg, total yield 7.5%), and a mixture M_2. M_1 (302 mg) was separated on a silica gel column using *n*-hexane−EtOAc (100:0 to 1:1) to yield **5** (25 mg, yield 0.7%). A similar fractionation of M_2 (589 mg) yielded **2** (54 mg), a 5:6 mixture of **2** and **3** (223 mg, ^{1}H NMR integration), and **3** (135 mg, total yield 7.6%).

Preparation of (*R*)- and (*S*)-MTPA Esters of 2 and 3. To a solution of **2** or **3** (2 mg in 0.5 mL of pyridine) was added *S*-(+)- or *R*-(−)-MTPA chloride (one drop), and the solution was allowed to stand at room temperature for 7 h. After purification using preparative TLC, the ester (1.8 mg, 90% yield) was analyzed by ^{1}H NMR spectroscopic measurement, and $\Delta\delta = \delta_S - \delta_R$ was calculated separately for **2** and **3**.

(1*R*,2*R*,4a*S*,8a*S*,3′*E*,5′*S*,1″*S*)-(+)-1-[6′-(2″,2″-Dimethyl-6″-methylenecyclohexyl)-4′-methyl-5′-hydroxyhex-3′-enyl]-2,5,5,8a-tetramethyldecahydronaphthalen-2-ol (2): colorless crystals; mp 145 °C; $[\alpha]_D$ +20 (*c* 0.2, CH_2Cl_2); IR (CH_2Cl_2) ν_{max} 3420 (OH), 2928 (CH), 1647 (double bond), 1124 (C−O) cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.71 (1H, m, H-1a), 1.67 (1H, m, H-1b), 1.54 (2H, m, H-2), 1.39 (1H, m, H-3a), 1.16 (1H, m, H-3b), 0.87 (1H, m, H-5), 1.58 (1H, m, H-6a), 1.42 (1H, m, H-6b), 1.86 (2H, m, H-7), 1.03 (1H, m, H-9), 1.47 (1H, m, H-11a), 1.33 (1H, m, H-11b), 2.07 (2H, m, H-12), 5.39 (1H, t, $J = 6.6$ Hz, H-13), 3.91 (1H, dd, $J = 9.6, 0.9$ Hz, H-15), 2.05 (1H, m, H-16a), 1.59 (1H, m, H-16b), 2.03 (1H, m, H-17), 1.48 (1H, m, H-19a), 1.25 (1H, m, H-19b), 1.67 (1H, m, H-20a), 1.24 (1H, m, H-20b), 2.03 (2H, m, H-21), 0.77 (3H, s, H-23), 0.84 (3H, s, H-24), 0.77 (3H, s, H-25), 1.11 (3H, s, H-26), 1.60 (3H, s, H-27), 0.82 (3H, s, H-28), 0.92 (3H, s, H-29), 4.78 (1H, s, H-30a), 4.60 (1H, s, H-30b); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 39.7 (t, C-1), 23.6 (t, C-2), 42.0 (t, C-3), 33.2 (s, C-4), 56.1 (d, C-5), 18.4 (t, C-6), 44.4 (t, C-7), 74.1 (s, C-8), 61.2 (d, C-9), 39.1 (s, C-10), 25.2 (t, C-11), 30.8 (t, C-12), 125.7 (d, C-13), 138.1 (s, C-14), 75.1 (d, C-15), 32.5 (t, C-16), 49.8 (d, C-17), 34.6 (s, C-18), 36.2 (t, C-19), 20.5 (t, C-20), 32.5 (t, C-21), 149.5 (s, C-22), 15.3 (q, C-23), 33.3 (q, C-24), 21.4 (q, C-25), 23.8 (q, C-26), 12.2 (q, C-27), 26.1 (q, C-28), 28.3 (q, C-29), 109.2 (t, C-30); EIMS *m/z* 444 $[M]^+$; FABMS *m/z* 467 $[M + Na]^+$, 427 $[M - OH]^+$, 409 $[M - H_2O - OH]^+$; HRESIMS *m/z* 427.3940 (calcd for $C_{30}H_{51}O$, 427.3940).

**(1*R*,2*R*,4a*S*,8a*S*,3′*E*,5′*R*,1″*S*)-(−)-1-[6′-(2″,2″-Dimethyl-6″-methylenecyclohexyl)-4′-methyl-5′-hydroxyhex-3′-enyl]-2,5,5,8a-tetram-

ethyldecahydronaphthalen-2-ol (3): amorphous solid; $[\alpha]_D$ −21 (*c* 0.2, CH_2Cl_2); IR (CH_2Cl_2) ν_{max} 3420 (OH), 2938 (CH), 1647 (double bond), 1124 (C−O) cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.65 (2H, m, H-1), 1.54 (2H, m, H-2), 1.41 (2H, m, H-3), 0.95 (1H, m, H-5), 1.61 (1H, m, H-6a), 1.43 (1H, m, H-6b), 1.88 (2H, m, H-7), 1.04 (1H, m, H-9), 1.52 (1H, m, H-11a), 1.34 (1H, m, H-11b), 2.09 (2H, m, H-12), 5.23 (1H, t, *J* = 7.0 Hz, H-13), 4.48 (1H, br d, *J* = 8.9 Hz, H-15), 2.20 (1H, m, H-16a), 2.06 (1H, m, H-16b), 2.08 (1H, m, H-17), 1.72 (2H, m, H-19), 1.54 (1H, m, H-20a), 1.30 (1H, m, H-20b), 2.10 (2H, m, H-21), 0.77 (3H, s, H-23), 0.87 (3H, s, H-24), 0.78 (3H, s, H-25), 1.13 (3H, s, H-26), 1.72 (3H, s, H-27), 0.85 (3H, s, H-28), 0.95 (3H, s, H-29), 4.83 (1H, s, H-30a), 4.64 (1H, s, H-30b); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 39.9 (t, C-1), 23.8 (t, C-2), 42.0 (t, C-3), 33.5 (s, C-4), 56.1 (d, C-5), 18.6 (t, C-6), 44.6 (t, C-7), 74.3 (s, C-8), 60.9 (d, C-9), 39.1 (s, C-10), 25.8 (t, C-11), 29.8 (t, C-12), 127.2 (d, C-13), 138.0 (s, C-14), 67.7 (d, C-15), 30.3 (t, C-16), 49.9 (d, C-17), 34.7 (s, C-18), 36.2 (t, C-19), 20.6 (t, C-20), 32.2 (t, C-21), 149.8 (s, C-22), 15.4 (q, C-23), 33.3 (q, C-24), 21.6 (q, C-25), 24.0 (q, C-26), 18.2 (q, C-27), 26.4 (q, C-28), 28.4 (q, C-29), 109.3 (t, C-30); EIMS *m/z* 426 $[M - H_2O]^+$; FABMS *m/z* 409 $[M - H_2O - OH]^+$; HRESIMS *m/z* 427.3937 (calcd for $C_{30}H_{51}O$, 427.3940).

(1*R*,2*R*,4a*S*,8a*S*,3′*E*,1″*S*)-(+)-1-[6′-(2″,2″-Dimethyl-6″-methylenecyclohexyl)-4′-hydroxymethylhex-3′-enyl]-2,5,5,8a-tetramethyldecahydronaphthalen-2-ol (4): amorphous solid; $[\alpha]_D$ +20.4 (*c* 0.2, CH_2Cl_2); IR (CH_2Cl_2) ν_{max} 3420 (OH), 2928 (CH), 1646 (double bond), 1134 (C−O) cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.63 (2H, m, H-1), 1.54 (2H, m, H-2), 1.43 (2H, m, H-3), 0.95 (1H, m, H-5), 1.45 (2H, m, H-6), 1.87 (2H, m, H-7), 1.05 (1H, m, H-9), 1.30 (2H, m, H-11), 1.88 (2H, m, H-12), 5.45 (1H, t, *J* = 7.1 Hz, H-13), 2.17 (2H, m, H-15), 1.52 (2H, m, H-16), 1.68 (1H, m, H-17), 1.47 (1H, m, H-19a), 1.26 (1H, m, H-19b), 1.72 (2H, m, H-20), 2.07 (2H, m, H-21), 0.79 (3H, s, H-23), 0.87 (3H, s, H-24), 0.79 (3H, s, H-25), 1.12 (3H, s, H-26), 4.03 (2H, s, H-27), 0.83 (3H, s, H-28), 0.91 (3H, s, H-29), 4.78 (1H, s, H-30a), 4.59 (1H, s, H-30b); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 39.8 (t, C-1), 23.7 (t, C-2), 42.0 (t, C-3), 33.4 (s, C-4), 56.0 (d, C-5), 18.4 (t, C-6), 44.3 (t, C-7), 74.0 (s, C-8), 61.2 (d, C-9), 39.1 (s, C-10), 25.6 (t, C-11), 27.1 (t, C-12), 126.8 (d, C-13), 139.5 (s, C-14), 30.7 (t, C-15), 25.5 (t, C-16), 54.4 (d, C-17), 34.9 (s, C-18), 36.2 (t, C-19), 20.5 (t, C-20), 32.4 (t, C-21), 149.1 (s, C-22), 15.4 (q, C-23), 33.2 (q, C-24), 21.5 (q, C-25), 23.8 (q, C-26), 66.7 (t, C-27), 26.3 (q, C-28), 28.3 (q, C-29), 109.0 (t, C-30); EIMS *m/z* 426 $[M - H_2O]^+$; FABMS *m/z* 427 $[M - OH]^+$, 409 $[M - H_2O - OH]^+$; HRESIMS *m/z* 427.3943 (calcd for $C_{30}H_{51}O$, 427.3940).

(1*R*,2*R*,4a*S*,8a*S*,3′*E*,1″*S*)-(+)-1-[6′-(2″,2″-Dimethyl-6″-methylenecyclohexyl)-4′-methyl-5′-oxohex-3′-enyl]-2,5,5,8a-tetramethyldecahydronaphthalen-2-ol (5): amorphous solid; $[\alpha]_D$ +22.4 (*c* 0.2, CH_2Cl_2); UV λ_{max} (MeOH) (log ϵ) 238 (3.9), 272 (0.9) nm; IR (CH_2Cl_2) ν_{max} 3490 (OH), 2930 (CH), 1718 (C═O, ester), 1645 (double bond), 1083 (C−O), 738 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.65 (2H, m, H-1), 1.50 (2H, m, H-2), 1.46 (1H, m, H-3a), 1.37 (1H, m, H-3b), 0.91 (1H, m, H-5), 1.46 (2H, m, H-6), 2.00 (2H, m, H-7), 1.11 (1H, m, H-9), 1.27 (2H, m, H-11), 2.33 (2H, m, H-12), 6.69 (1H, t, *J* = 7.0 Hz, H-13), 2.85 (2H, m, H-16), 2.65 (1H, m, H-17), 1.55 (2H, m, H-19), 1.68 (2H, m, H-20), 2.05 (2H, m, H-21), 0.81 (3H, s, H-23), 0.88 (3H, s, H-24), 0.81 (3H, s, H-25), 1.18 (3H, s, H-26), 1.76 (3H, s, H-27), 0. 88 (3H, s, H-28), 0.95 (3H, s, H-29), 4.68 (1H, s, H-30a), 4.38 (1H, s, H-30b); ^{13}C NMR ($CDCl_3$, 75 MHz,) δ 39.9 (t, C-1), 24.1 (t, C-2), 42.0 (t, C-3), 33.3 (s, C-4), 56.2 (d, C-5), 18.4 (t, C-6), 44.8 (t, C-7), 74.3 (s, C-8), 61.4 (d, C-9), 39.1 (s, C-10), 23.9 (t, C-11), 35.3 (t, C-12), 142.2 (d, C-13), 137.4 (s, C-14), 201.8 (s, C-15), 34.5 (t, C-16), 49.2 (d, C-17), 35.0 (s, C-18), 38.7 (t, C-19), 20.6 (t, C-20), 32.4 (t, C-21), 149.5 (s, C-22), 15.4 (q, C-23), 33.4 (q, C-24), 21.6 (q, C-25), 23.7 (q, C-26), 12.0 (q, C-27), 24.5 (q, C-28), 28.9 (q, C-29), 108.2 (t, C-30); FABMS *m/z* 465 $[M + Na]^+$, 443 $[M + H]^+$; HRESIMS *m/z* 443.3886 (calcd for $C_{30}H_{51}O_2$, 443.3889).

Benzoylation of 2 and 3. A mixture of **2** and **3** (200 mg, 0.45 mmol) was treated with benzoyl chloride (0.1 mL) and pyridine (2 mL) at 50 °C for 15 h. The crude residue (287 mg) was separated on Sephadex LH-20 using CH_2Cl_2−MeOH (1:1) to give four fractions (F_1−F_4). Fraction F_2 (127 mg) was chromatographed using normal-phase HPLC and *n*-hexane−CH_2Cl_2−MeOH (8:8:1) to yield three fractions (A−C). Fraction B (55 mg) was further separated using normal-phase HPLC and *n*-hexane−EtOAc (12:1) to yield **6** (24 mg, yield 9.7%) and **7** (12 mg, yield 4.9%).

(1*R*,2*R*,4a*S*,8a*S*,3′*E*,5′*S*,1″*S*)-(+)-1-[6′-(2″,2″-Dimethyl-6″-methylenecyclohexyl)-4′-methyl-5′-benzoyloxyhex-3′-enyl]-2,5,5,8a-tetramethyldecahydronaphthalen-2-ol (6): amorphous solid; $[\alpha]_D$ +60.4 (*c* 0.2, CH_2Cl_2); UV λ_{max} (MeOH) (log ϵ) 238 (4.53), 272 (3.39) nm; IR (CH_2Cl_2) ν_{max} 3502 (OH), 2933, 2866 (CH), 1718 (C═O, ester), 1646 (double bond), 1276 (C−O, ester), 1118 (C−O), 711 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.75 (2H, m, H-1), 1.60 (2H, m, H-2), 1.35 (1H, m, H-3a), 1.16 (1H, m, H-3b), 0.84 (1H, m, H-5), 1.60 (1H, m, H-6a), 1.42 (1H, m, H-6b), 1.88 (2H, m, H-7), 1.08 (1H, m, H-9), 1.42 (2H, m, H-11), 2.56 (1H, m, H-12a), 2.13 (1H, m, H-12b), 5.26 (1H, t, *J* = 6.2 Hz, H-13), 5.73 (1H, dd, *J* = 9.0, 1.5 Hz, H-15), 2.10 (1H, m, H-16a), 1.60 (1H, m, H-16b), 1.96 (1H, m, H-17), 1.51 (1H, m, H-19a), 1.26 (1H, m, H-19b), 1.63 (1H, m, H-20a), 1.30 (1H, m, H-20b), 2.10 (2H, m, H-21), 0.77 (3H, s, H-23), 0.80 (3H, s, H-24), 0.77 (3H, s, H-25), 1.16 (3H, s, H-26), 1.72 (3H, s, H-27), 0.90 (3H, s, H-28), 0.94 (3H, s, H-29), 4.82 (1H, s, H-30a), 4.50 (1H, s, H-30b), 8.07 (2H, d, *J* = 7.5 Hz, H-3′,7′), 7.46 (2H, t, *J* = 7.5 Hz, H-4′,6′), 7.57 (1H, t, *J* = 7.5 Hz, H-5′); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 39.9 (t, C-1), 23.6 (t, C-2), 42.1 (t, C-3), 33.4 (s, C-4), 56.0 (d, C-5), 18.6 (t, C-6), 44.6 (t, C-7), 74.2 (s, C-8), 60.0 (d, C-9), 39.0 (s, C-10), 25.0 (t, C-11), 30.1 (t, C-12), 129.7 (d, C-13), 133.6 (s, C-14), 72.6 (d, C-15), 30.5 (t, C-16), 50.0 (d, C-17), 34.7 (s, C-18), 36.2 (t, C-19), 20.7 (t, C-20), 32.5 (t, C-21), 148.4 (s, C-22), 15.5 (q, C-23), 33.4 (q, C-24), 21.6 (q, C-25), 23.6 (q, C-26), 18.4 (q, C-27), 26.3 (q, C-28), 28.4 (q, C-29), 110.1 (t, C-30), 165.9 (s, C-1′), 130.8 (s, C-2′), 129.7 (d, C-3′,7′), 128.4 (d, C-4′,6′), 132.8 (d, C-5′); EIMS *m/z* 426; FABMS *m/z* 531 $[M - OH]^+$, 409 $[M - PhCOOH - OH]^+$; HRESIMS *m/z* 531.4201 (calcd for $C_{37}H_{55}O_2$, 531.4202).

(1*R*,2*R*,4a*S*,8a*S*,3′*E*,5′*R*,1″*S*)-(+)-1-[6′-(2″,2″-Dimethyl-6″-methylenecyclohexyl)-4′-methyl-5′-benzoyloxyhex-3′-enyl]-2,5,5,8a-tetramethyldecahydronaphthalen-2-ol (7): amorphous solid; $[\alpha]_D$ −20.3 (*c* 0.2, CH_2Cl_2); UV λ_{max} (MeOH) (log ϵ) 238 (4.51), 273 (3.13) nm; IR (CH_2Cl_2) ν_{max} 3502 (OH), 2932 (CH), 1715 (C═O, ester), 1646 (double bond), 1601 (aromatic), 1276 (C−O, ester), 1113 (C−O), 711 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.72 (2H, m, H-1), 1.56 (2H, m, H-2), 1.38 (1H, m, H-3a), 1.14 (1H, m, H-3b), 0.89 (1H, m, H-5), 1.46 (2H, m, H-6), 1.88 (2H, m, H-7), 1.07 (1H, m, H-9), 1.44 (2H, m, H-11), 2.20 (2H, m, H-12), 5.55 (1H, t, *J* = 7.3 Hz, H-13), 5.38 (1H, t, *J* = 7.2 Hz, H-15), 1.93 (2H, m, H-16), 1.75 (1H, m, H-17), 1.54 (1H, m, H-19a), 1.33 (1H, m, H-19b), 1.71 (2H, m, H-20), 2.25 (1H, m, H-21a), 2.02 (1H, m, H-21b), 0.78 (3H, s, H-23), 0.85 (3H, s, H-24), 0.78 (3H, s, H-25), 1.14 (3H, s, H-26), 1.70 (3H, s, H-27), 0.85 (3H, s, H-28), 0.93 (3H, s, H-29), 4.82 (1H, s, H-30a), 4.72 (1H, s, H-30b), 8.04 (2H, d, *J* = 7.2 Hz, H-3′,7′), 7.43 (2H, t, *J* = 7.2 Hz, H-4′,6′), 7.54 (1H, t, *J* = 7.2 Hz, H-5′); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 39.8 (t, C-1), 23.8 (t, C-2), 41.9 (t, C-3), 33.2 (s, C-4), 56.1 (d, C-5), 18.4 (t, C-6), 44.7 (t, C-7), 74.1 (s, C-8), 61.3 (d, C-9), 39.1 (s, C-10), 25.0 (t, C-11), 31.0 (t, C-12), 131.4 (d, C-13), 131.5 (s, C-14), 80.4 (d, C-15), 28.7 (t, C-16), 50.1 (d, C-17), 35.0 (s, C-18), 37.2 (t, C-19), 20.6 (t, C-20), 33.2 (t, C-21), 148.4 (s, C-22), 15.4 (q, C-23), 33.4 (q, C-24), 21.5 (q, C-25), 23.8 (q, C-26), 11.3 (q, C-27), 25.3 (q, C-28), 28.5 (q, C-29), 109.4 (t, C-30), 165.7 (s, C-1′), 131.1 (s, C-2′), 129.5 (d, C-3′,7′), 128.2 (d, C-4′,6′), 132.6 (d, C-5′); EIMS *m/z* 426, 408; FABMS *m/z* 531 $[M - OH]^+$; HRESIMS *m/z* 531.4200 (calcd for $C_{37}H_{55}O_2$, 531.4202).

Benzoylation of 4. Compound **4** (60 mg, 0.14 mmol) was subjected to benzoylation, and chromatographic purification of the product using the same methods mentioned under benzoylation of **2** and **3**, to yield **8** (9 mg, yield 12.2%).

(1*R*,2*R*,4a*S*,8a*S*,3′*E*,1″*S*)-(+)-1-[6′-(2″,2″-Dimethyl-6″-methylenecyclohexyl)-4′-benzoyloxymethylhex-3′-enyl]-2,5,5,8a-tetramethyldecahydronaphthalen-2-ol (8): amorphous solid; $[\alpha]_D$ +23 (*c* 0.2, CH_2Cl_2); UV λ_{max} (MeOH) (log ϵ) 229 nm (4.36); IR (CH_2Cl_2) ν_{max} 3450 (OH), 2932 (CH), 1716 (C═O, ester), 1646 (double bond), 1605 (aromatic), 1270 (C−O, ester), 1111 (C−O), 711 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.64 (2H, m, H-1), 1.58 (2H, m, H-2), 1.40 (1H, m, H-3a), 1.14 (1H, m, H-3b), 0.97 (1H, m, H-5), 1.62 (1H, m, H-6a), 1.42 (1H, m, H-6b), 1.40 (1H, m, H-7a), 1.18 (1H, m, H-7b), 1.08 (1H, m, H-9), 1.58 (2H, m, H-11), 2.01 (2H, m, H-12), 5.60 (1H, t, *J* = 5.3 Hz, H-13), 2.13 (2H, m, H-15), 1.35 (2H, m, H-16), 1.73 (1H, m, H-17), 1.52 (1H, m, H-19a), 1.26 (1H, m, H-19b), 1.73 (2H, m, H-20), 2.05 (2H, m, H-21), 0.79 (3H, s, H-23), 0.86 (3H, s, H-24), 0.79 (3H, s, H-25), 1.14 (3H, s, H-26), 4.75 (2H, s, H-27), 0.82 (3H, s, H-28), 0.90 (3H, s, H-29), 4.75 (1H, s, H-30a), 4.58 (1H, s, H-30b), 8.07 (2H, d, *J* = 7.6 Hz, H-3′,7′), 7.44 (2H, t, *J* = 7.6 Hz, H-4′,6′), 7.54 (1H, t, J = 7.6 Hz, H-5′); ^{13}C NMR (CDCl$_3$, 75 MHz) δ 39.8 (t, C-1), 23.6 (t, C-2), 42.0 (t, C-3), 33.2 (s, C-4), 56.1 (d, C-5), 18.5 (t, C-6), 44.6 (t, C-7), 74.0 (s, C-8), 61.5 (d, C-9), 39.1 (s, C-10), 25.5 (t, C-11), 27.5 (t, C-12), 128.3 (d, C-13), 134.5 (s, C-14), 31.0 (t, C-15), 25.3 (t, C-16), 54.4 (d, C-17), 34.8 (s, C-18), 36.1 (t, C-19), 20.6 (t, C-20), 32.3 (t, C-21), 149.1 (s, C-22), 15.4 (q, C-23), 33.4 (q, C-24), 21.5 (q, C-25), 23.8 (q, C-26), 69.5 (t, C-27), 26.3 (q, C-28), 28.3 (q, C-29), 109.1 (t, C-30), 166.5 (s, C-1′), 130.9 (s, C-2′), 129.6 (d, C-3′,7′), 128.3 (d, C-4′,6′), 132.8 (d, C-5′); EIMS *m/z* 309 [M − PhCOOH − OH]$^+$; FABMS *m/z* 571 [M + Na]$^+$; HRESIMS *m/z* 571.4130 (calcd for $C_{37}H_{56}O_3Na$, 571.4127).

Reaction with *p*-Toluenesulfonyl Chloride. A mixture of **3** (100 mg, 0.45 mmol), *p*-toluenesulfonyl chloride (0.1 mL), and pyridine (1.5 mL) was stirred at 50 °C for 12 h. Ether was added, and the mixture was washed with saturated aqueous NaHCO$_3$ solution and brine, dried, and evaporated. The resulting crude residue (127 mg) was chromatographed on a silica gel column using *n*-hexane−EtOAc (100:0 to 10:1) to afford two fractions (A and B). Fraction A (89 mg) was separated by silica gel preparative TLC (GF$_{254}$) using *n*-hexane−CH$_2$Cl$_2$ (3:1) to yield **9** (20 mg, 20.8%) and **10** (42 mg, 43.8%).

(1*R*,2*R*,4a*S*,8a*S*,3′*R*,4′*E*,1″*S*)-(+)-1-[6′-(2″,2″-Dimethyl-6″-methylenecyclohexyl)-4′-methylhex-4′-enyl]-2,3′-epoxy-2,5,5,8a-tetramethyldecahydronaphthalen-2-ol (9): colorless, needle-shaped crystals; mp 112 °C; [α]$_D$ +25.2 (*c* 0.2, CH$_2$Cl$_2$); IR (CH$_2$Cl$_2$) ν_{max} 3400 (OH), 2928 (CH), 1646 (double bond), 1074 (C−O) cm^{-1}; ^{1}H NMR (CDCl$_3$, 300 MHz) δ 1.54 (2H, m, H-1), 1.53 (2H, m, H-2), 1.35 (2H, m, H-3), 0.96 (1H, m, H-5), 1.42 (2H, m, H-6), 1.83 (2H, m, H-7), 1.60 (1H, m, H-9), 1.79 (1H, m, H-11a), 1.55 (1H, m, H-11b), 1.61 (2H, m, H-12), 4.12 (1H, dd, J = 9.9, 1.2 Hz, H-13), 5.34 (1H, t, J = 6.6 Hz, H-15), 2.22 (1H, m, H-16a), 2.08 (1H, m, H-16b), 1.79 (1H, m, H-17), 1.46 (1H, m, H-19a), 1.26 (1H, m, H-19b), 1.60 (2H, m, H-20), 2.06 (1H, m, H-21a), 2.00 (1H, m, H-21b), 0.80 (3H, s, H-23), 0.86 (3H, s, H-24), 0.80 (3H, s, H-25), 1.21 (3H, s, H-26), 1.60 (3H, s, H-27), 0.83 (3H, s, H-28), 0.93 (3H, s, H-29), 4.74 (1H, s, H-30a), 4.50 (1H, s, H-30b); ^{13}C NMR (CDCl$_3$, 75 MHz) δ 39.1 (t, C-1), 23.7 (t, C-2), 42.1 (t, C-3), 33.3 (s, C-4), 56.4 (d, C-5), 18.5 (t, C-6), 43.5 (t, C-7), 74.9 (s, C-8), 51.9 (d, C-9), 37.1 (s, C-10), 26.4 (t, C-11), 15.9 (t, C-12), 75.6 (d, C-13), 136.3 (s, C-14), 125.7 (d, C-15), 24.9 (t, C-16), 54.0 (d, C-17), 35.0 (s, C-18), 37.2 (t, C-19), 20.2 (t, C-20), 33.2 (t, C-21), 149.4 (s, C-22), 15.0 (q, C-23), 33.4 (q, C-24), 21.5 (q, C-25), 25.9 (q, C-26), 12.4 (q, C-27), 25.3 (q, C-28), 28.6 (q, C-29), 108.8 (t, C-30); EIMS *m/z* 409 [M − OH]$^+$; FABMS *m/z* 449 [M + Na]$^+$, 427 [M + H]$^+$; HRESIMS *m/z* 427.3941 (calcd for $C_{30}H_{51}O$, 427.3940).

Crystal data for 9: $C_{30}H_{50}O_1$, *M* 426.72, orthorhombic, $P2_12_12_1$ (No. 19), *a* 6.298(2) Å, *b* 11.916(2) Å, *c* 35.057(5) Å, *V* 2631.1(7) Å^3, *Z* 4, D_{calcd} 1.077 g cm^{-3}, *F*(000) 952, λ(Mo Kα) 0.71073 Å, *T* 298 K, 2232 unique reflections collected. Final GooF 1.35, *R* 0.0476, *wR* 0.0705, 280 parameters, μ 0.062 mm^{-1}, *R* indices based on 1546 reflections with $I > 2\sigma(I)$, absorption corrections applied. Copies of the deposited crystal data can be obtained, free of charge, on application to CCDC (file number 626138), 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44(0) 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk].

(1*R*,2*R*,4a*S*,8a*S*,5′*S*,3′*E*,1″*S*)-(+)-1-[6′-(2″,2″-Dimethyl-6″-methylenecyclohexyl)-4′-methylhex-3′-enyl]-2,5′-epoxy-2,5,5,8a-tetramethyldecahydronaphthalen-2-ol (10): colorless, needle-shaped crystals; mp 98 °C; [α]$_D$ −24.3 (*c* 0.2, CH$_2$Cl$_2$); IR (CH$_2$Cl$_2$) ν_{max} 3400 (OH), 2928 (CH), 1647 (double bond), 1078 (C−O) cm^{-1}; ^{1}H NMR (CDCl$_3$, 300 MHz) δ 1.65 (1H, m, H-1a), 1.60 (1H, m, H-1b), 1.54 (2H, m, H-2), 1.44 (1H, m, H-3a), 1.40 (1H, m, H-3b), 0.99 (1H, m, H-5), 1.44 (2H, m, H-6), 1.80 (2H, m, H-7), 1.20 (1H, m, H-9), 2.27 (1H, m, H-11a), 2.10 (1H, m, H-11b), 1.60 (1H, m, H-12a), 1.49 (1H, m, H-12b), 5.28 (1H, br t, J = 6.2 Hz, H-13), 3.96 (1H, dd, J = 9.2, 0.9 Hz, H-15), 1.62 (2H, m, H-16), 1.84 (1H, m, H-17), 1.50 (1H, m, H-19a), 1.31 (1H, m, H-19b), 1.76 (2H, m, H-20), 2.15 (1H, m, H-21a), 2.00 (1H, m, H-21b), 0.74 (3H, s, H-23), 0.86 (3H, s, H-24), 0.80 (3H, s, H-25), 1.25 (3H, s, H-26), 1.60 (3H, s, H-27), 0.81 (3H, s, H-28), 0.93 (3H, s, H-29), 4.74 (1H, s, H-30a), 4.47 (1H, s, H-30b); ^{13}C NMR (CDCl$_3$, 75 MHz) δ 39.1 (t, C-1), 23.8 (t, C-2), 42.0 (t, C-3), 33.3 (s, C-4), 56.4 (d, C-5), 18.6 (t, C-6), 42.2 (t, C-7), 74.6 (s, C-8), 57.4 (d, C-9), 36.8 (s, C-10), 24.7 (t, C-11), 31.8 (t, C-12), 126.4 (d, C-13), 135.9 (s, C-14), 75.5 (d, C-15), 18.4 (t, C-16), 53.7 (d, C-17), 35.1 (s, C-18), 37.7 (t, C-19), 19.9 (t, C-20), 33.8 (t, C-21), 149.3 (s, C-22), 15.6 (q, C-23), 33.4 (q, C-24), 21.3 (q, C-25), 20.7 (q, C-26), 12.0 (q, C-27), 24.7 (q, C-28), 28.8 (q, C-29), 108.7 (t, C-30); EIMS *m/z* 409 [M − OH]$^+$; FABMS *m/z* 449 [M + Na]$^+$, 427 [M + H]$^+$; HRESIMS *m/z* 427.3942 (calcd for $C_{30}H_{51}O$, 427.3940).

Photoreaction of Ambrein (1). Ambrein (**1**, 300 mg) was dissolved in acetone (5 mL) containing a few drops of H$_2$O and irradiated with shortwave UV light (254 nm, UV lamp) for 72 h at room temperature. The mixture was stirred continuously, and the evaporated solvent was replaced. The solution was then evaporated, and the residue (285 mg) was separated on a silica gel column using *n*-hexane−EtOAc (100:1 to 2:1) to afford **12** (6 mg) and **11** (25 mg, 8.3%).

(1*R*,2*R*,4a*S*,8a*S*,3′*S*,4′*S*,1″*S*)-(+)-1-[6′-(2″,2″-Dimethyl-6″-methylenecyclohexyl)-4′-hydroxy-4′-methylhexyl]-2,3′-epoxy-2,5,5,8a-tetramethyldecahydronaphthalen-2-ol (11): amorphous solid; [α]$_D$ −19.8 (*c* 0.2, CH$_2$Cl$_2$); IR (CH$_2$Cl$_2$) ν_{max} 3446 (OH), 2926 (CH), 1645 (double bond), 1088 (C−O) cm^{-1}; ^{1}H NMR (CDCl$_3$, 300 MHz) δ 1.57 (2H, m, H-1), 1.54 (2H, m, H-2), 1.36 (2H, m, H-3), 1.00 (1H, m, H-5), 1.50 (2H, m, H-6), 1.79 (2H, m, H-7), 1.15 (1H, m, H-9), 1.65 (2H, m, H-11), 1.65 (2H, m, H-12), 3.38 (1H, dd, J = 9.9, 2.0 Hz, H-13), 2.06 (2H, m, H-15), 1.48 (2H, m, H-16), 1.65 (1H, m, H-17), 1.53 (2H, m, H-19), 1.21 (2H, m, H-20), 2.00 (2H, m, H-21), 0.74 (3H, s, H-23), 0.87 (3H, s, H-24), 0.80 (3H, s, H-25), 1.26 (3H, s, H-26), 1.11 (3H, s, H-27), 0.92 (3H, s, H-28), 0.85 (3H, s, H-29), 4.75 (1H, brs, H-30a), 4.56 (1H, brs, H-30b); ^{13}C NMR (CDCl$_3$, 75 MHz) δ 39.1 (t, C-1), 23.9 (t, C-2), 42.1 (t, C-3), 33.3 (s, C-4), 57.6 (d, C-5), 18.6 (t, C-6), 41.9 (t, C-7), 73.3 (s, C-8), 56.4 (d, C-9), 36.7 (s, C-10), 26.6 (t, C-11), 19.9 (t, C-12), 75.2 (d, C-13), 74.9 (s, C-14), 36.5 (t, C-15), 18.2 (t, C-16), 54.7 (d, C-17), 35.1 (s, C-18), 35.4 (t, C-19), 20.7 (t, C-20), 32.7 (t, C-21), 149.4 (s, C-22), 15.6 (q, C-23), 33.3 (q, C-24), 21.3 (q, C-25), 29.7 (q, C-26), 23.7 (q, C-27), 26.1 (q, C-28), 28.5 (q, C-29), 108.9 (t, C-30); FABMS *m/z* 467 [M + Na]$^+$; HRESIMS *m/z* 467.3865 (calcd for $C_{30}H_{52}O_2Na$, 467.3865).

Cytotoxicity Assay. Cytotoxicity was determined against Hepa59T/VGH (human liver carcinoma), WiDr (human colon adenocarcinoma), A-549 (human lung carcinoma), and MCF-7 (human breast adenocarcinoma) tumor cells was based on a MTT assay method. The assay procedure was carried out as previously described.[17] ED$_{50}$ values were defined by a comparison with the untreated cells as the concentration of test sample resulting in 50% reduction of absorbance.

Anti-inflammatory Assay (Measurement of Elastase Release). Degranulation of azurophilic granules was determined by elastase release as described previously,[18] with some modifications. Experiments were performed using MeO-Suc-Ala-Ala-Pro-Val-*p*-nitroanilide as the elastase substrate. In brief, after supplementation with MeO-Suc-Ala-Ala-Pro-Val-*p*-nitroanilide (100 μM), neutrophils (6 × 10^5/mL) were equilibrated at 37 °C for 2 min and incubated with each test compound for 5 min. Cells were activated by FMLP (100 nM)/CB (0.5 μg/mL), and changes in absorbance at 405 nm were monitored continuously for elastase release. The results are expressed as the percentage of the initial rate of elastase release in the FMLP/CB-activated, test compound-free (DMSO) control system.

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