Deoxyartemisinin Derivatives from Photooxygenation of Anhydrodeoxydihydroartemisinin and Their Cytotoxic Evaluation

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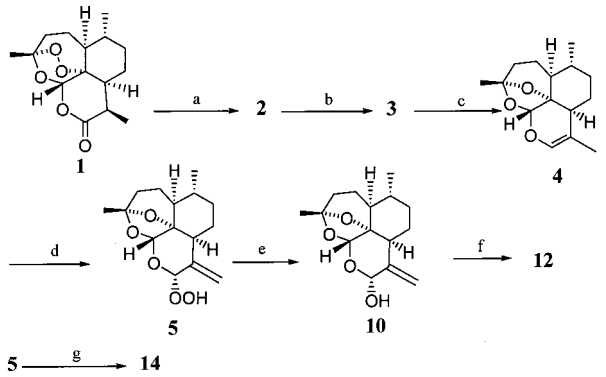
Photooxygenation of anhydrodeoxydihydroartemisinin (**4**) followed by chromatographic separation of the reaction mixture yielded the new compounds α- (**5**) and *β*-hydroperoxydeoxyartemisitene (**8**) and the formate ester **7**, together with two previously reported compounds, **6** and **9**. Reduction of **5** using polymer-bound triphenylphosphine afforded the new compound dihydrodeoxyartemisitene (**10**). Treatment of **10** with a catalytic amount of BF_3-OEt_2 yielded the C_2-symmetrical dimer bis(dihydrodeoxyartemisitene) ether (**11**) and two new compounds, dihydrodeoxyartemisitene methyl ether (**12**) and the dimer **13**, as minor products. Dehydroacetoxylation of **5** using acetic anhydride in pyridine afforded deoxyartemisitene (**14**). The identities of the new compounds (**5**, **7**, **8**, **10**−**14**) were deduced from their spectral data and by chemical derivatization. The stereochemistry of dimer **11** was defined on the basis of X-ray crystallographic analysis. All compounds were evaluated in vitro in the National Cancer Institute drug-screening program consisting of 60 human cancer cell lines derived from nine different tissues. Of the compounds tested, deoxyartemisitene (**14**) demonstrated significant cytotoxicity against a number of human cancer cell lines.

Previous reports on artemisinin derivatives with a peroxy functionality, including those with a $\Delta^{11(13)}$ exo-methylene moiety, documented significant cytotoxicity for several cancer cell lines.[1] Reports on cytotoxicity of the deoxyartemisinin derivatives are scarce; however, some have shown antitumor activity.[2,3] On the other hand, there are no reports on the cytotoxicity of deoxyartemisitene derivatives. In this study, several deoxyartemisitene compounds were prepared, and their cytotoxicity was evaluated in an in vitro cytotoxicity screen provided by the National Cancer Institute, Bethesda, MD.

Results and Discussion

The key compound anhydrodeoxydihydroartemisinin (**4**) was prepared from artemisinin (**1**) by reduction with sodium borohydride to give dihydroartemisinin (**2**) followed by hydrogenation to afford deoxydihydroartemisinin (**3**),[4] which was then converted to **4**[5] with BF_3-OEt_2 (Scheme 1). Compound **4** was then subjected to photochemical oxygenation using *meso*-tetraphenylporphine as a photosensitizer.[6] The major product of photooxygenation, the new compound **5**, was obtained in 55% yield by crystallization from hexanes−EtOAc. The mother liquor from crystallization of **5** was chromatographed on a Si gel column to yield the new compound **7** in addition to the known compounds **6** and **9.** Reduction of **5** using polymer-bound triphenylphosphine yielded compound **10**. Compound **10** was transformed by BF_3-OEt_2 into the new dimeric compound **11**, as well as two further new compounds, **12** and **13**, that were formed as side products. Dehydroacetoxylation of **5** using Ac_2O in pyridine yielded the new compound deoxyartemisitene (**14**) (87% yield) (Scheme 1).

Scheme 1. Synthesis of Deoxyartemisinin Derivatives[a]



[a] (a) MeOH, $NaBH_4$, 0−5 °C, 3 h; (b) $Pd/CaCO_3$, H_2, room temperature, atmospheric pressure; (c) BF_3-OEt_2, hexane-diethyl ether (1:1), room temperature; (d) *meso*-tetraphenylporphine, O_2, light, CH_2Cl_2-Me_2CO, 2.5 h; (e) Polymer-bound triphenylphosphine, CH_2Cl_2; (f) BF_3-OEt_2, diethyl ether; (g) Ac_2O, pyr.

Compound **5** displayed a deprotonated molecular ion peak in the HRESIMS at *m/z* 281.1429, supporting a molecular formula of $C_{15}H_{22}O_5$. The presence of an exo-methylene group was indicated by signals in the ^{13}C NMR spectrum at δ 142.0 (C-11) and 114.5 (C-13) and in the ^1H NMR spectrum by two olefinic protons resonating at δ 5.33 (s) and 5.11 (s). The presence of two methyl groups, C-14 and C-15, was evident from signals in the ^{13}C and ^1H NMR spectra at δ 18.5 and 0.91 (d) and at δ 23.6 and 1.50 (s), respectively. The ^1H NMR spectrum also showed three characteristic proton signals at δ 5.52 (s), 2.59 (dd), and 5.82 (s) assigned to H-5, H-7, and H-12, respectively. The presence of an OOH group was evident from the appearance of an IR peak at ν_{max} 3362 cm^{-1} and the observation of a ^1H NMR peak at δ 9.54 (1H, brs, exchangeable). The relative stereochemistry of the hydroperoxide group at C-12 in **5** was determined as α on the basis of NOESY data,

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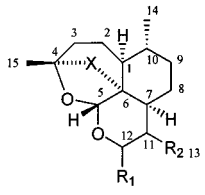
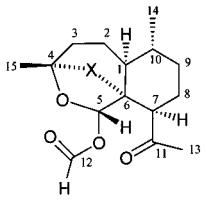
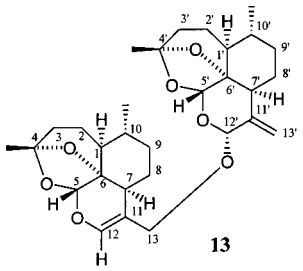


	X	R_1	R_2
1	O-O	=O	β-CH_3
2	O-O	OH	β-CH_3
3	O	OH	β-CH_3
8	O	β-OOH	=CH_2
12	O	β-Ome	=CH_2
14	O	=O	=CH_2
16	O-O	=O	=CH_2



7 X = O
15 X = O-O



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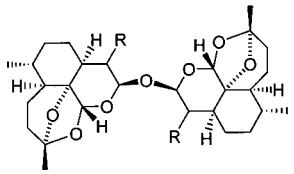
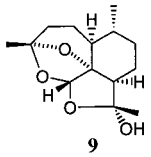


6 R = α-CH_3
11 R = =CH_2



9

which showed correlations of proton H-12 with both the *β*-oriented Me-15 and proton H-5. The above data were consistent with structure **5**.

Compound **7** had a molecular formula of $C_{15}H_{22}O_5$ based on HRESIMS. Its ^{13}C and ^1H NMR spectral data (Table 1 and Experimental Section) suggested that it is the deoxy analogue of the known compound **15**.[7] The ^{13}C NMR spectrum of **7** showed the presence of a ketone carbonyl functionality resonating at *δ* 207.6, a formyl carbonyl at *δ* 159.7, and three methyl groups at *δ* 18.7, 24.2, and 32.0. The ^1H NMR spectrum supported the presence of a formyl residue with a proton at *δ* 7.87 and three methyl signals at *δ* 0.91 (d), 1.56 (s), and 2.36 (s), with the latter being attached to a carbonyl group of a ketone. In the IR spectrum, absorption bands corresponding to the formyl ester and keto group were observed at ν_{max} 1745 and 1710 cm^{-1}, respectively. The foregoing evidence was used to confirm structure **7**, which was further verified by conversion to compound **9**[8] and by preparation from compound **15**.[9]

Examination of the ^1H (Experimental Section) and ^{13}C NMR (Table 1) data as well as the mass spectrum indicated that **8** is an epimer of **5** with the OOH group attached to C-12 being *β*-oriented, which was also supported by the change of optical rotation ($[\alpha]_D$ −102.3°).

Analysis of the physical and spectral data of compound **6** revealed that it has been reported previously.[5,10] Compound **9** was also found to be of known chemical structure on the basis of comparison of its spectral data with literature values.[8]

Reduction of **5** using polymer-bound triphenylphosphine[9] yielded **10**. The ^1H and ^{13}C NMR spectral data indicated that it was the expected deoxy analogue of dihydroartemisitene.[9] The HRESIMS of **10** displayed a molecular ion peak at *m*/*z* 265.1482 $[M - H]^-$ corresponding to a molecular formula of $C_{15}H_{22}O_4$. The IR spectrum showed a hydroxyl absorption band at ν_{max} 3340 cm^{-1}. The relative stereochemistry of the hydroxyl group at C-12 in **10** was determined as α on the basis of the mechanism of reduction of **5** to **10**, which proceeds with retention of stereochemistry, and this assignment was supported by NOESY NMR data, which showed correlations of H-12 with the *β*-oriented Me-15. Although the C-12 α isomer predominated, there was ample evidence in the ^1H NMR spectrum for the presence of the C-12*β* diastereomer, albeit in a low concentration (approximately in the ratio of 1:4). Thus, the aforementioned data were in agreement with the assignment of **10** as dihydrodeoxyartemisitene.

The C_2-symmetrical dimer **11**, obtained via $BF_3{-}OEt_2$-catalyzed dimerization of **10**, showed a molecular ion peak at *m*/*z* 537.2815 $[M + Na]^+$, corresponding to the molecular formula $C_{30}H_{42}O_7$. The ^1H (Experimental Section) and ^{13}C NMR spectral data (Table 1) of **11** were consistent with the structure shown. X-ray crystallographic analysis not only confirmed this assignment but also established the relative stereochemistry of the acetal linkage at C-12 as *β*. The asymmetric crystal unit consists of two molecules of **11** with overall very similar conformations. A view of the solid-state conformation of one of these molecules is presented in Figure 1. Corresponding bond distances in the two independent molecules of **11** as well as those in the individual molecules agree well and all lie close to expected

Table 1. ^{13}C NMR Chemical Shift Assignments[a] for Compounds **4**, **5**, **7**, **8**, **10**−**12**, and **14**

carbon	**4**	**5**	**7**	**8**	**10**	**11**[b]	**12**	**14**
1	45.7, d	44.2, d	47.1, d	44.8, d	44.3, d	44.5, d	44.5, d	44.4, d
2	21.6, t	21.9, t	22.2, t	21.8, t	21.9, t	22.0, t	22.0, t	22.0, t
3	34.4, t	34.3, t	33.8, t	34.8, t	34.4, t	34.5, t	34.5, t	33.9, t
4	107.7, s	107.8, s	109.6, s	108.9, s	107.4, s	107.5, s	107.3, s	109.8, s
5	95.6, d	96.8, d	92.9, d	95.3, d	97.2, d	97.2, d	96.0, d	99.4, d
6	83.3, s	81.6, s	87.1, s	80.9, s	81.3, s	81.6, s	81.4, s	82.3, s
7	41.1, d	45.1, d	54.7, d	43.5, d	44.4, d	44.4, d	44.6, d	44.7, d
8	27.6, t	32.6, t	24.2, t	32.1, t	33.4, t	33.3, t	33.1, t	30.9, t
9	34.2, t	34.0, t	33.7, t	33.9, t	34.1, t	34.2, t	34.1, t	33.6, t
10	35.3, d	35.3, d	35.3, d	35.2, d	35.2, d	35.2, d	35.3, d	35.5, d
11	112.6, s	142.0, s	207.6, s	140.5, s	148.5, s	145.0, s	145.5, s	135.5, s
12	133.9, d	99.0, d	159.7, s	100.7,d	89.4, d	92.8, d	97.1, d	162.9, s
13	16.6, q	114.5, t	32.0, t	119.3, t	112.6, t	112.6, t	112.7, t	129.1, t
14	18.8, q	18.5, q	18.7, q	18.5, q	18.5, q	18.6, q	18.6, q	18.5, q
15	24.2, q	23.6, q	24.2, q	23.2, q	23.6, q	23.1, q	23.7, q	24.0, q
OMe							56.0	

[a] In $CDCl_3$ at 75 MHz. Carbon multiplicities were determined by DEPT 135 experiments. [b] These same values are assigned also to the corresponding carbons (1′−15′) of the other half of dimer **11**.

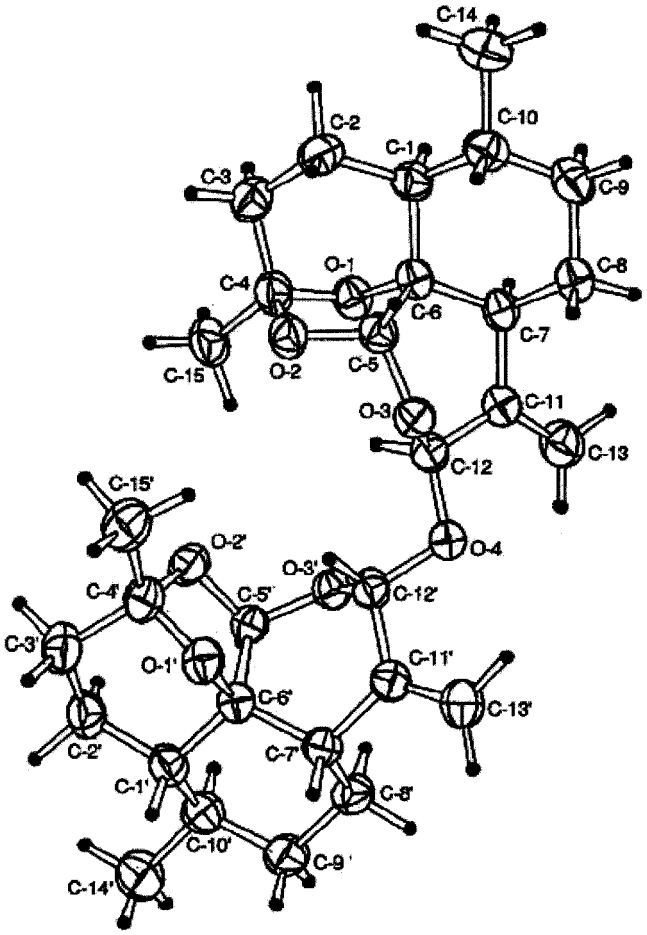


Figure 1. ORTEP diagram (40% probability ellipsoids) showing the crystallographic atom-numbering scheme and solid-state conformation of one of the molecules of **11** in the asymmetric crystal unit; small filled circles represent hydrogen atoms.

values.[11] The conformations of like rings are also similar and did not differ significantly from those in a C_2-symmetrical dimer prepared earlier by treating deoxydihydroartemisinin with *p*-toluenesulfonic acid in dry toluene.[5,10]

Fractionation of the mother liquor of **11** using column chromatography yielded **12** and **13** as colorless solids. The ^{1}H and ^{13}C NMR spectra indicated that **12** is the C-12 *O*-methyl derivative of **10** with a methoxy group attached to C-12. The methoxy signal appeared at δ 56.0 in the ^{13}C NMR spectrum and at δ 3.52 in the ^{1}H NMR spectrum. The HRESIMS supported the assigned structure **12** by displaying a sodiated molecular ion at *m/z* 303.1581, corresponding to the molecular formula $C_{16}H_{24}O_4$. The stereochemistry of the methoxy group at C-12 was determined as β on the basis of its NOESY correlation with the β-oriented H-5. Structure **12** was further confirmed by its preparation from **10** by acetalization with methanol in the presence of a catalytic amount of BF_3-OEt_2. This compound is apparently an artifact formed during crystallization of **11** using a mixture of hexane−EtOAc−MeOH. It is worth noting that compound **12** was formed with the C-12 methoxy group exclusively in the β-orientation.

The HRESIMS of compound **13** showed a molecular ion at *m/z* 537.2822 $[M + Na]^+$, indicating a molecular formula of $C_{30}H_{42}O_7$. The ^{13}C NMR spectrum of **13** (Table 1) exhibited 30 carbon signals with close similarity to **4** and **5** (Experimental Section), which in combination with the HRESIMS data indicated that **13** is an unsymmetrical dimer. Inspection of the ^{13}C NMR spectrum also revealed

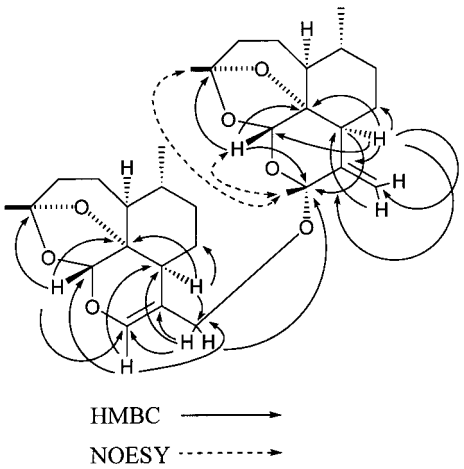


Figure 2. Important HMBC and NOESY correlations of **13**.

the existence of an exomethylene functionality with two carbon signals resonating at δ 112.6 (C-13′) and 146.1 (C-11′), an olefinic moeity with a methine carbon signal at δ 140.6 (C-12), and a quaternary carbon signal at δ 112.4 (C-11), in addition to the characteristic allylic oxymethylene signal at δ 68.5. The ^{1}H NMR data supported the presence of three olefinic protons, two belonging to the exomethylene functionality at δ 5.00 (H-13′b) and 5.27 (H-13′a) and the third proton belonging to the methine carbon at δ 6.39 (H-12). The two protons of the methylene group at C-13 appeared as doublets at δ 4.25 (1H, d, J = 12.2 Hz) and 4.20 (1 H, d, J = 12.2 Hz). Confirmation was made by analysis of the HMQC and HMBC spectra of **13** (Figure 2). The relative stereochemistry at C-12′ was defined on the basis of a NOESY experiment in which H-12′ displayed correlations with the β-oriented Me-15′ and H-5′, and accordingly, the acetal linkage was assigned with an α-orientation.

Dehydroacetoxylation of **5** by treatment with acetic anhydride in pyridine [7] furnished **14**. The ^{13}C NMR spectrum of **14** exhibited a characteristic lactone carbonyl signal at δ 162.9. The IR absorption band at ν_{max} 1740 cm^{-1} confirmed the presence of an α,β-unsaturated lactone moiety. The HRESIMS showed a molecular ion peak at *m/z* 263.1260 $[M - H]^-$ corresponding to a molecular formula of $C_{15}H_{20}O_4$. Thus, spectral data interpretation, in addition to the comparison of the spectral data of the peroxy analogue artemisitene (**16**),[12] supported the assignment of compound **14** as deoxyartemisitene.

All compounds were tested in vitro at National Cancer Institute using their panel of 60 human tumor cell lines.[13] Deoxyartemisitene (**14**) demonstrated considerable cytotoxicity against a number of human cancer cell lines. Its cytotoxicity was comparable to that of artemisitene (**16**).[14] Deoxyartemisitene (**14**) displayed some selectivity toward leukemia. In the leukemia subpanel, it was active against the HL-60 (TB), CCFR-CEM, and K-562 cell lines, with ED_{50} values of 0.69, 0.92, and 0.94 μg/mL, respectively, as well as the RPMT-8226 and SR cell lines, with ED_{50} values of 1.68 and 1.89 μg/mL, respectively. It also showed cytotoxicity against the ovarian cancer cell line IGROVI and the non-small cell lung cancer cell line (HOP-92) with ED_{50} values of 0.89 and 0.92 μg/mL, respectively. In addition, cytotoxic activity was observed against the MCF7 and B7-549 breast cancer cell lines with ED_{50} values of 1.46 and 1.97 μg/mL, respectively.

Experimental Section

General Experimental Procedures. Melting points were recorded on an Electrothermal 9100 instrument. Optical

rotations were recorded at ambient temperature using a JASCO DIP 370 digital polarimeter. IR spectra were obtained using a ATI Mattson Genesis Series FTIR spectrometer. The ^{1}H NMR spectra were recorded in $CDCl_3$ on a Varian VXR 300 instrument at 300 MHz (^{1}H) and 75 MHz (^{13}C) or a Bruker DRX 400 spectrometer operating at 400 MHz for ^{1}H and 100 MHz ^{13}C, using the solvent peak as reference. 2D NMR spectra were measured with standard pulse programs and acquisition parameters. Mass spectra were recorded on a Finnigan MAT 300 mass spectrometer using methane as ionization gas. HRESIMS were obtained on a Bruker BioAPEX 30es ion cyclotron high-resolution HPLC-FT spectrometer by direct injection into an electrospray interface. TLC was performed on precoated Si gel G plates (E. Merck) using mixtures of EtOAc and hexane as solvent and visualized by spraying with *p*-anisaldehyde spray reagent.[15] Artemisinin (**1**) was isolated from *Artemisia annua* L. (Asteraceae) plants grown in Saudi Arabia, following a literature procedure.[16]

Photooxygenation of 4. Compound **4** (1.9 g) was introduced into dudly tubes and dissolved in CH_2Cl_2−acetone (1:1, 25 mL). The photosensitization dye *meso*-tetraphenylporphine was added (5 mg) to each tube, and the solution acquired a wine-red color. The reaction mixture was then subjected to 650 W incandescent light while a stream of oxygen was bubbled gently through it, and its temperature was maintained at 25 °C. After 2.5 h, the solvent was distilled off from the reaction mixture to leave a red-colored solid. The colored solid was washed with ether (3 × 100 mL) to remove the lipophilic dye. The resulting colorless solid showing a single spot on TLC with R_f 0.43 (toluene−EtOAc, 8:2) was crystallized from hexane−EtOAc to yield colorless prisms of **5** (1.04 g, 55%): mp 193−194 °C, $[\alpha]_D$ +27.0° (*c* 0.026, $CHCl_3$); IR (KBr) ν_{max} 3362 cm^{-1} (OOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.54 (1H, brs, OOH), 5.82 (1H, s, H-12), 5.52 (1H, s, H-5), 5.33 (1H, s, H-13a), 5.11 (1H, s, H-13b), 2.59 (1H, dd, J = 12.8, 4.5 Hz, H-7), 1.88 (1H, m, H-2a), 1.74−1.66 (3H, m, H-3a, H-8a, H-9a), 1.58 (2H, m, H-3b and H-8b), 1.50 (3H, s, Me-15), 1.30−1.11 (4H, m, H-1, H-2b, H-9b, H-10), 0.91 (3H, d, J = 5.5 Hz, Me-14); ^{13}C NMR (Table 1); HRESIMS m/z 281.1429 $[M - H]^-$ (calcd for $C_{15}H_{21}O_5$, 281.1394).

The mother liquor left after crystallization of **5** was concentrated under reduced pressure to give an orange oily residue (0.850 g). This residue was dissolved in a small volume of hexane−ether (1:1) and chromatographed on a Si gel column (85 g). Elution with 10% ether in hexane yielded **6** (54 mg, colorless crystals). Compound **6** had physical and spectral data that were indistinguishable from the reported values.[5,10] Further elution with 10% ether in hexane provided **7** (54.7 mg, R_f 0.53, hexane−EtOAc, 7:3): prisms (hexane−EtOAc); mp 193−194 °C $[\alpha]_D$ −170.8° (*c* 0.11, $CHCl_3$); IR (KBr) ν_{max} 1745, 1710 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.87 (1H, s, H-12), 6.37 (1H, s, H-5), 2.36 (3H, s, Me-13), 2.58 (1H, dd, J = 12.8, 3.3 Hz, H-7), 2.00 (1H, m, H-8a), 1.89 (1H, m, H-2a), 1.77 (2H, m, H-3a, H-9a), 1.64 (1H, m, H-3b, 1.56 (3H, s, Me-15), 1.39−1.29 (3H, m, H-2b, H-8b, H-10), 1.20 (1H, m, H-1), 1.04 (1H, m, H-9b), 0.91 (3H, d, J = 5.8 Hz, Me-14); ^{13}C NMR (Table 1); HRESIMS m/z 281.1426 $[M - H]^-$ (calcd for $C_{15}H_{21}O_5$, 281.1389).

Further elution with 10% ether in hexane afforded **8**. Compound **8** was obtained as a gum (68 mg): R_f 0.32 (hexane−EtOAc, 7:3); $[\alpha]_D$ −102.3° (*c* 0.20, $CHCl_3$); IR (KBr) ν_{max} 3424, cm^{-1}; ^{1}HNMR δ 0.92 (3H, d, J = 5.4 Hz, Me-14), 1.29−1.16 (4H, m, H-1, H-2, H-9, H-10), 1.54 (1H, m, H-8), 1.55 (1H, s, Me-15), 1.61 (1H, m, H-3), 1.72−1.64 (3H, m, H-3′, H-8′, H-9′), 1.88 (1H, m, H-2′), 2.61 (1H, dd, J = 13.0, 4.7 Hz, H-7), 5.30 (1H, brs, H-13), 5.32 (1H, brs, H-13′), 5.39 (1H, s, H-5), 5.67 (1H, brt, J = 1.3 Hz, H-12), 9.19 (1H, s, OOH); ^{13}C NMR (Table 1); EIMS m/z 282 [M$^+$] (6), 249 (6), 235 (9), 221 (13), 218 (10), 194 (16), 176 (17), 165 (33), 151 (37), 149 (19), 131 (13), and 44 (100).

Further elution with 10% ether in hexane yielded **9** (85 mg, R_f 0.37, hexane−ether, 1:1), which was found to be identical to a previously reported compound.[8]

Preparation of Compound 10. Deoxyhydroperoxide **5** (100 mg) was dissolved by stirring in dry CH_2Cl_2 (7 mL) at room temperature. Polymer-bound triphenylphosphine (200 mg) was added, and the reaction mixture was stirred for 50 min and filtered, and the residue was washed with CH_2Cl_2. Evaporation of the solvent furnished **10** as a colorless solid (86 mg, 92%, R_f 0.19, hexane−EtOAc, 8:2): needles (hexane−EtOAc); mp 184−185 °C; $[\alpha]_D$ +20.3° (*c* 0.06, $CHCl_3$); IR (KBr) ν_{max} 3340 cm^{-1} (OH); ^{1}H NMR ($CDCl_3$, 400 MHz), δ 5.63 (1H, s, J = 9.0 Hz, H-12), 5.45 (1H, s, H-5), 5.38 (1H, s, H-13a), 5.07 (1H, s, H-13b), 3.18 (1H, brs, exchangeable, OH-12), 2.62 (1H, dd, J = 12.6, 5.1 Hz, H-7), 1.87 (1H, m, H-2a), 1.75−1.65 (3H, m, H-3a, H-8a, H-9a), 1.60 (1H, m, H-8b), 1.53 (1H, m, H-3b), 1.49 (3H, s, Me-15), 1.28−1.11 (4H, m, H-1, H-2b, H-9b, H-10), 0.91 (3H, d, J = 5.3 Hz, Me-14); ^{13}C NMR (Table 1); HRESIMS m/z 265.1482 $[M - H]^-$ (calcd for $C_{15}H_{21}O_4$, 265.1445).

Dimerization of 10. Deoxydihydroartemisitene **10** (250 mg) was dissolved in dry ether (125 mL, 0 °C), and BF_3−OEt_2 (0.25 mL) was added while the solution was stirred. Additional BF_3−OEt_2 (0.25 mL each) was added after 35 and 60 min. The temperature was raised to 10 °C (30 min), then to room temperature, where it was stirred for 12 h before quenching by addition of 5 mL of 2% aqueous solution of $NaHCO_3$. The mixture was diluted with 250 mL of ether and washed with water, and the ether phase was separated and dried over anhydrous Na_2SO_4. Evaporation of ether afforded a yellowish oil, which, upon crystallization, yielded **11** (200 mg, 41.5%, R_f 0.52, hexane−EtOAc, 8:2): colorless prisms (hexane−EtOAc-MeOH); mp 295−296 °C; $[\alpha]_D$ +17.0° (*c* 0.11, $CHCl_3$); IR (KBr) no OH absorption band; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.73 (1H, s, H-12), 5.48 (1H, brs, H-5), 5.41 (1H, brs, H-13a), 5.09 (1H, brs, H-13b), 2.62 (1H, dd, J = 12.8, 5.2 Hz, H-7), 1.80−1.57 (6H, m, H-3a, H-3b, H-8a, H-8b, H-9a, and H-2a), 1.53 (3H, s, Me-15), 1.28−1.13 (4H, m, H-1, H-2b, H-9b, and H-10), 0.90 (3H, d, J = 5.1 Hz, Me-14); ^{13}C NMR (Table 1); HRESIMS m/z 537.2815 $[M + Na]^+$ (calcd for $C_{30}H_{42}O_7Na$, 537.2817).

The mother liquor from the crystallization of compound **11** was concentrated under reduced pressure to leave a gummy residue (96 mg). The residue was flash-chromatographed on a Si gel 60 column (10 g) using 10% EtOAc in hexane to afford **12** (20 mg, R_f 0.52, hexanes−EtOAc, 8:2): amorphous solid; $[\alpha]_D$ +16.0° (*c* 0.086, $CHCl_3$); IR (KBr) no OH absorption band; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.48 (1H, s, H-12), 5.30 (1H, s, H-13a), 5.28 (1H, s, H-5), 5.05 (1H, s, H-13b), 3.52 (3H, s, OMe-12), 2.59 (1H, dd, J = 12.7, 5.2 Hz, H-7), 1.87 (1H, m, H-2a), 1.75−1.50 (5H, m, H-3a, H-3b, H-8a, H-8b, and H-9a), 1.50 (3H, s, Me-15), 1.27−1.22 (4H, m, H-1, H-2b, H-9b, and H-10), 0.91 (3H, d, J = 5.7 Hz, Me-14); ^{13}C NMR (Table 1); HRESIMS m/z 303.1581 $[M + Na]^+$ (calcd for $C_{16}H_{24}O_4Na$, 303.1566).

Further elution with 10% EtOAc in hexane afforded dimer **13** (20 mg, colorless solid, R_f 0.36, hexanes−EtOAc, 8:2): $[\alpha]_D$ +8.3° (*c* 0.024, $CHCl_3$); IR (film) ν_{max} 2938, 2872, 1673, 1455, 1386, 1266, 1006 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.39 (1H, s, H-12), 5.51 (1H, s, H-5), 5.45 (1H, s, H-5′), 5.41 (1H, s, H-12′), 5.27 (1H, brs, H-13′a), 5.00 (1H, brs, H-13′b), 4.25 (1H, d, J = 12.2 Hz), 4.20 (1H, d, J = 12.2 Hz) (CH_2-13), 2.59 (1H, dd, J = 12.7, 4.2 Hz, H-7′), 2.21 (1H, dd, J = 12.5, 4.2 Hz, H-7), 1.92−1.81 (4H, m, H-2a, H-2b, H-2′a, H-2′b), 1.72−1.53 (10 H, H-8a, H-8b, H-9a, H-9b, H-9′a, H-9′b, H-3a, H-3b, H-3′a, H-3′b), 1.47 (3H, s, Me-15′), 1.44 (3H, s, Me-15), 1.24 (4H, m, H-8′a, H-8′b, H-1, H-1′), 1.11 (2H, m, H-10, H-10′), 0.90 (6H, J = 4.9 Hz, Me-14, Me-14′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 146.1 (s, C-11′), 140.6 (d, C-12), 112.6 (s, C-13′), 112.4 (t, C-11), 108.4 (s, C-4), 107.5 (s, C-4′), 97.4 (d, C-5′), 96.4 (d, C-5), 91.6 (d, C-12′), 84.2 (s, C-6′), 68.5 (t, C-13), 45.7 (d, C-1), 44.8 (d, C-7′)[a], 44.7 (d, C-1′)[a], 39.3 (d, C-7), 35.5 (d, C-10)[b], 35.4 (d, C-10′)[b], 34.8 (t, C-3), 34.4 (t, C-3′), 34.3 (t, C-9, C-9′), 33.6 (t, C-8′), 29.4 (t, C-8), 24.4 (q, C-15′), 23.9 (q, C-15), 22.0 (t, C-2), 22.4 (t, C-2′), 19.0 (q, C-14), 18.8 (q, C-14′) (*a* and *b*, assignments may be reversed); HRESIMS m/z 537.2822 $[M + Na]^+$ (calcd for $C_{30}H_{42}O_7Na$, 537.2810).

Dehydroacetoxylation of 5. The hydroperoxide **5** (50 mg) was dissolved in Ac_2O (0.5 mL) containing 0.1 mL of pyridine. The solution was stirred at room temperature for 50 min and

then was worked up by addition of $CHCl_3$ (50 mL), washing with 2% aqueous $NaHCO_3$ solution (2 mL), followed by 2% HCl (2 mL), and finally with H_2O (10 mL). The $CHCl_3$ extract was dried over anhydrous Na_2SO_4. Removal of the solvent yielded **14** (41.0 mg, 87%, R_f 0.60, toluene−EtOAc, 8:2): prisms (CH_2Cl_2−EtOAc); mp 188−189 °C; $[\alpha]_D$ −86.3° (*c* 0.04, $CHCl_3$); IR (KBr) ν_{max} 1740 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ6.41 (1H, d, J = 1.2 Hz, H-13a), 5.78 (1H, s, H-5), 5.63 (1H, brs, H-13b), 2.79 (1H, dd, J = 13.3, 4.6 Hz, H-7) 1.81−1.72 (4H, m, H-2a, H-3a, H-8a, H-9a), 1.56 (2H, m, H-3b and H-8b), 1.48 (3H, s, Me-15), 1.33−1.22 (4H, m, H-1, H-2b, H-9b, and H-10), 0.95 (3H, d, J = 5.8 Hz, Me-14); ^{13}C NMR (Table 1); HRESIMS *m*/*z* 263.1260 $[M - H]^-$ (calcd for $C_{15}H_{20}O_4$, 263.1288).

Conversion of 10 to 12. To a stirred solution of **10** (5 mg) in dry ether (2 mL) was added MeOH (0.15 mL), followed by BF_3−OEt_2 (9.0 μL). Stirring was continued for 30 min, then the reaction was quenched and worked up as before to give **12** (3.0 mg, 57%).

Conversion of 7 to 9. Compound **7** (33 mg) was dissolved in absolute ethanol (8 mL) and stirred at 0 °C until a clear solution was obtained, and $NaBH_4$ (16.3 mg) was then added. Stirring was continued for 1.5 h, then the reaction was quenched by the addition of acetic acid (0.1 mL) and worked up as usual to produce an oily residue. This residue was subjected to column chromatography on Si gel using hexane−EtOAc (8:2) as eluent to afford **9** (20 mg, 76%) as a colorless solid, with physical and spectral data indistinguishable from isolated **9**.

Conversion of 15 to 7. Compound **15** (38 mg) was dissolved in MeOH (15 mL), and Pd on $CaCO_3$ (10 mg) was added. The mixture was subjected to hydrogenation under atmospheric pressure for 7 h at room temperature. The reaction mixture was then worked up as usual to produce **7** (32.0 mg, 90%), with physical and spectral data indistinguishable from isolated **7**.

X-ray Crystal Structure Analysis of Compound 11.[17] Crystal data: $C_{30}H_{42}O_7$; MW = 514.67, monoclinic, space group $P2_1(C_2^2)$, a = 20.063(3) Å, b = 13.307(2) Å, c = 10.571(2) Å, β = 103.17(1)°, V = 2748(1) Å^3, Z = 4, D_c = 1.244 g cm^{-3}, μ(Cu Kα radiation, λ = 1.5418 Å) = 6.7 cm^{-1}; crystal dimensions 0.30 × 0.30 × 0.56 mm.

An Enraf-Nonius CAD-4 diffractometer (Cu Kα radiation, graphite monochromator) was used for all X-ray measurements. The space group was determined from the Laue symmetry, systematic absences ($0k0$ when $k \neq 2n$), and the fact that **11** is chiral.

Refined unit-cell parameters were calculated from the diffractometer setting angles for 25 reflections ($37° < \theta < 40°$) widely separated in reciprocal space. Intensity data ($+h$, $+k$, $\pm l$, θ_{max} = 75°, 5919 nonequivalent reflections), recorded at 298 K by ω−2θ scans [scanwidths (0.80 + 0.14 tan θ)°], were corrected for the usual Lorentz and polarization effects; an empirical absorption correction, based on the ϕ-dependency of the intensities of several reflections with χ ca. 90°, was also applied [$T_{max.}$:$T_{min.}$(relative) = 1.00:0.92]. Four reference reflections, remeasured at 2 h intervals throughout the data collection, showed no significant variation (<1%).

The crystal structure was solved by direct methods. The enantiomer was chosen to yield the configuration consistent with precursor **4**. Atomic positional and thermal parameters of the carbon and oxygen atoms (first isotropic and then anisotropic) were adjusted by means of several rounds of full-matrix least-squares calculations during which $\sum w\Delta^2$[w = $1/\sigma^2(|F_o|)$, $\Delta = (|F_o| - |F_c|)$] was minimized; hydrogen atoms were incorporated at their calculated positions during the later cycles. An extinction correction (g) was included as a variable during the final iterations (total number of parameters = 667), which converged (max. shift:esd = 0.03) at R = 0.039, R_w = 0.056, GOF = 1.48, $g = 1.8(1) \times 10^{-6}$ {$R = \sum||F_o| - |F_c||/\sum|F_o|$, $R_w = [\sum w(|F_o| - |F_c|)^2/\sum w|F_o|^2]^{1/2}$, GOF = $[\sum w\Delta^2/(N_{observns} - N_{param})]^{1/2}$} over 5149 reflections with $I > 2.0\sigma(I)$. No unusual features were present in a final difference Fourier synthesis [$\Delta\rho$(e/A^3) = 0.19(max), −0.18(min)].

Crystallographic calculations were performed by use of the Enraf-Nonius Structure Determination Package (SDP 3.0).[18] For all structure-factor calculations, neutral atom scattering factors and their anomalous dispersion corrections were taken from the literature.[19]

Tables of crystallographic data have been deposited with the Cambridge Crystallographic Data Centre. Copies of these data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44(0)-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

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