Tandem Silica Gel-Catalysed Rearrangements and Subsequent Baeyer–Villiger Reactions of Artemisinin Derivatives

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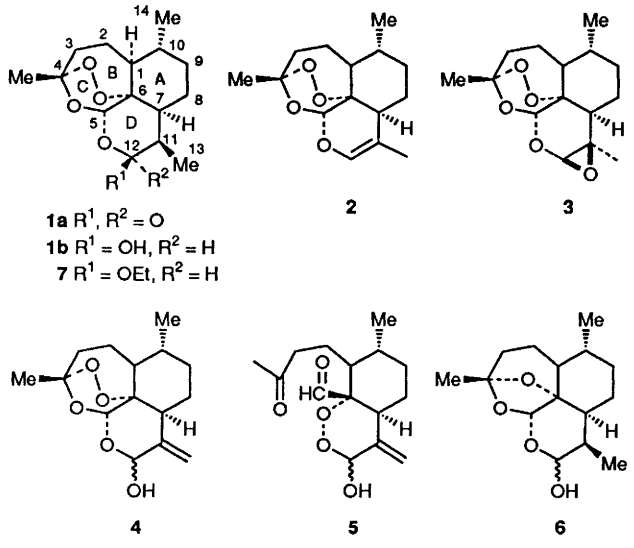
Novel silica gel-catalysed reactions of dihydroartemisinin **1b** to deoxyartemisinin **11** and 11β-hydroxy-11-epidihydroartemisinin **8** to compound **9** under mild conditions are described. The structures of the products were determined by mass spectrometry and 1D- and 2D-NMR spectroscopy. A mechanism for their formation is proposed.

Isolation, by Chinese investigators, of artemisinin **1a** (Fig. 1) and their demonstration that it was effective in treating patients infected with drug-resistant strains of *Plasmodium falciparum*,



1a R^1, R^2 = O
1b R^1 = OH, R^2 = H
7 R^1 = OEt, R^2 = H

2 **3** **4** **5** **6**

Fig. 1 Artemisinin **1a** and related compounds

even those with cerebral malaria, prompted structure–activity investigations of artemisinin derivatives.[1] These studies established that the labile peroxide moiety was required for antimalarial activity.[1a,b] Many derivatives[1b] of dihydroartemisinin **1b** were prepared by converting the free hydroxy group into a variety of ethers, esters, carbonates, urethanes, *etc.* In searching for new derivatives several investigators prepared anhydrodihydroartemisinin **2** from compound **1b** and attempted to convert the double bond into an epoxide **3**. When Lin *et al.*[2a] treated compound **2** with *m*-chloroperbenzoic acid (MCPBA) they found that the epoxides reacted with *m*-chlorobenzoic acid, formed in the reaction, to yield a mixture of two hydroxy esters. Later, Petrov and Ognyanov[2b] successfully isolated the 11β,12β-epoxide **3** by reaction of compound **2** with a complex of MCPBA and KF.

In order to prepare 11β-hydroxy-11-epidihydroartemisinin **8**, an aqueous acetone solution of epoxide **3** was treated with dil. aq. sulfuric acid. The resulting diol, however, proved surprisingly difficult to purify by silica gel chromatography. During chromatography diol **8** underwent an unusual silica gel-catalysed rearrangement and Baeyer–Villiger reaction to form a less polar compound **9**. The structure of compound **9** has been determined and a mechanism for its formation is suggested. The reactions of dihydroartemisinin **1b** and 11α-hydroxydihydroartemisinin **10** with silica gel under similar conditions were investigated and the structure of a product obtained from compound **1b** was identified.

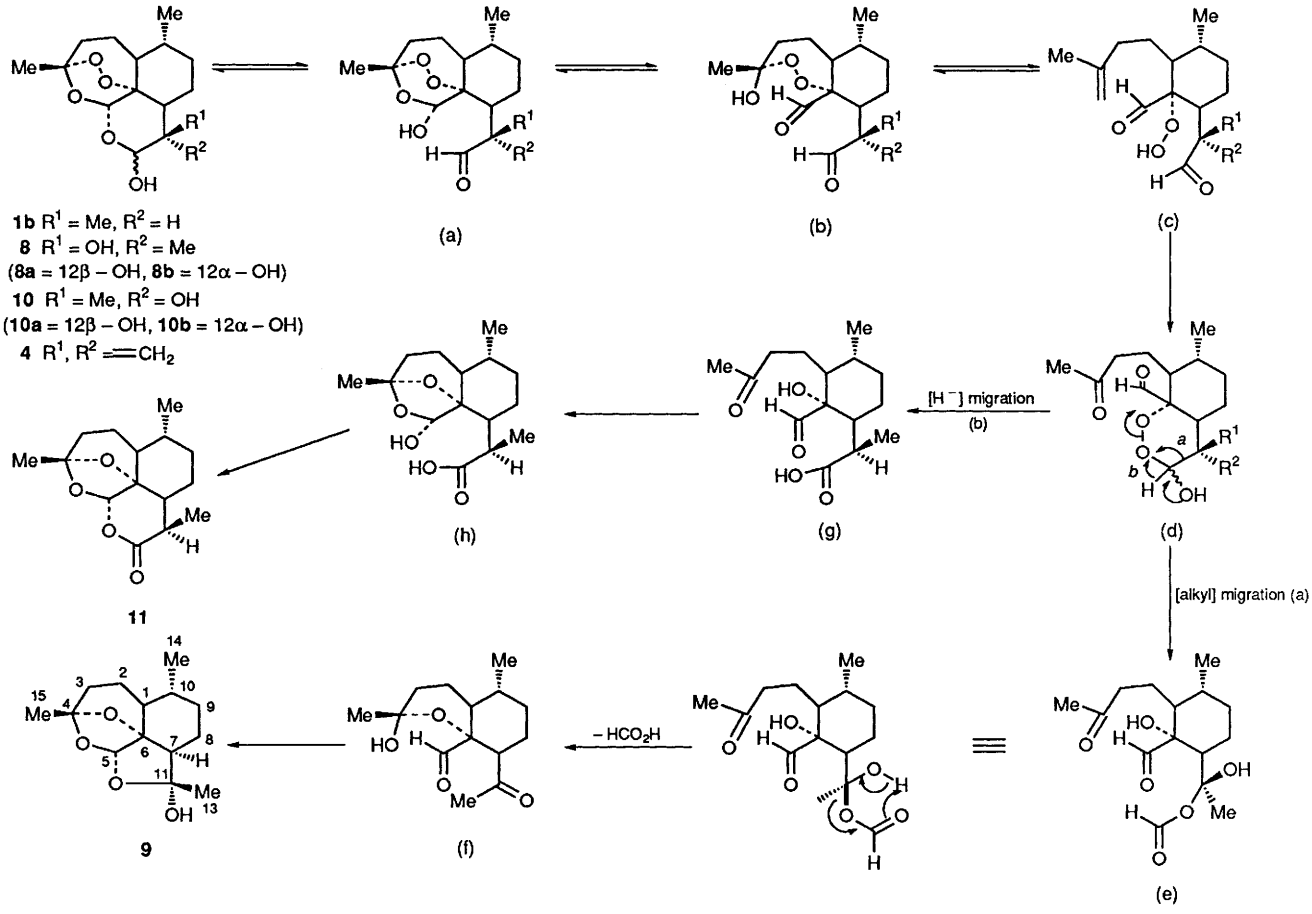
Results and discussion

Treatment of crude epoxide **3**, prepared as described by Petrov and Ognyanov,[2b] with ethanol and toluene-*p*-sulfonic acid yielded a mixture of hydroxy ethers, which were separated by chromatography. Analysis of their ^{1}H NMR spectra indicated they were epimeric at C-11, analogous to the isomeric 11-hydroxy-12β-*O*-(*m*-chlorobenzoyl)dihydroartemisinins prepared by Lin *et al.*[2a] These observations suggested that both the 11α,12α- and the 11β,12β-epoxide were present in the crude reaction mixture. A careful examination of the ^{1}H NMR spectrum of the crude reaction mixture revealed that it contained 20–25% of the 11α,12α-epoxide. In attempting to separate the mixture of epoxides by chromatography on silica gel, we found that the α-epoxide reacted with water more rapidly than did the β-isomer. A pure sample of β-epoxide **3** was obtained from the crude epoxide mixture by flash chromatography over silica gel. During flash chromatography, the 11α,12α-epoxide was selectively converted into 11α-hydroxydihydroartemisinin **10**, which was eluted after epoxide **3** with more polar solvent mixtures.

Treatment of epoxide **3** with aq. acetone containing a trace of sulfuric acid yielded 11β-hydroxy-11-epidihydroartemisinin **8**, which was purified by flash chromatography. If diol **8** remained in contact with silica gel it was slowly converted into the rearranged product **9**. Investigation of the conditions that convert diol **8** into compound **9** revealed that the reaction was catalysed by silica gel in propan-1-ol, chloroform or (most rapidly) benzene. A 70% yield of compound **9** was obtained by refluxing a benzene solution of diol **8** with silica gel for 10 min.

Molecular-ion measurements by EI and CI mass spectrometry with a variety of gases, showed diol **8** lost CH_2O_2 in its conversion into compound **9**. The latter's structure and stereochemistry were deduced from the following spectroscopic data. The ^{1}H NMR spectrum showed the presence of three methyl signals: a doublet at δ 0.96 (10-Me) and singlets at 1.35 (11-Me) and 1.58 (15-Me), and a single proton at 5.65 (5-H). There was no signal observed for 12-H. The ^{13}C NMR spectrum of compound **9** showed 14 carbons, *i.e.* the loss of one carbon signal in agreement with mass spectral data. Tentative assignments of the ^{13}C resonances in compound **9** were made (see Table 1) from distortionless enhancement by polarisation transfer (DEPT) and 2D heteronuclear multiple quantum coherence (HMQC) spectra. The assignments were verified by an analysis of the 2D heteronuclear multiple bond coherence (HMBC) spectrum (Fig. 2).

The absence, in the ^{13}C NMR spectrum, of the C-12 resonance in compound **9** and that of 12-H in the ^{1}H NMR



Scheme 1 Proposed mechanism for silica gel-catalysed transformation of the artemisinin derivatives and subsequent Baeyer–Villiger reactions

Table 1 ^{13}C NMR Spectral data and assignments of compounds **8** (**8a** + **8b**), **10** (**10a** + **10b**) and **9**

	8[a]		**10**[a]		
Carbon	**8a**	**8b**	**10a**	**10b**	**9**
1	52.24	52.02	52.29	51.37	42.19
2	24.50	24.42	25.47	25.05	23.37
3	36.49	36.33	36.36	36.24	34.59
4	103.5	104.4	104.3	104.5	111.2
5	88.57	91.66	87.35	90.82	103.4
6	81.80	82.00	83.44	83.01	94.18
7	50.54	50.62	49.17	49.47	49.93
8	23.31	22.96	24.51	24.51	26.25
9	34.08	33.97	34.37	33.95	32.27
10	37.42	37.42	37.38	37.38	35.36
11	70.74	71.30	70.97	71.85	110.7
12	96.09	93.66	100.1	93.81	
13	28.12	29.66	22.66	22.05	21.88
14	20.05	20.19	20.34	20.23	18.64
15	25.68	25.53	25.89	25.89	24.66

[a]The special assignments were made from a mixture of diastereoisomeric hemiacetals in equilibrium.

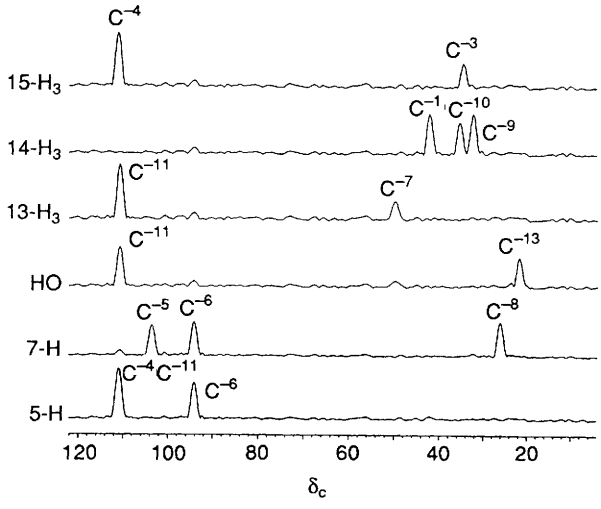
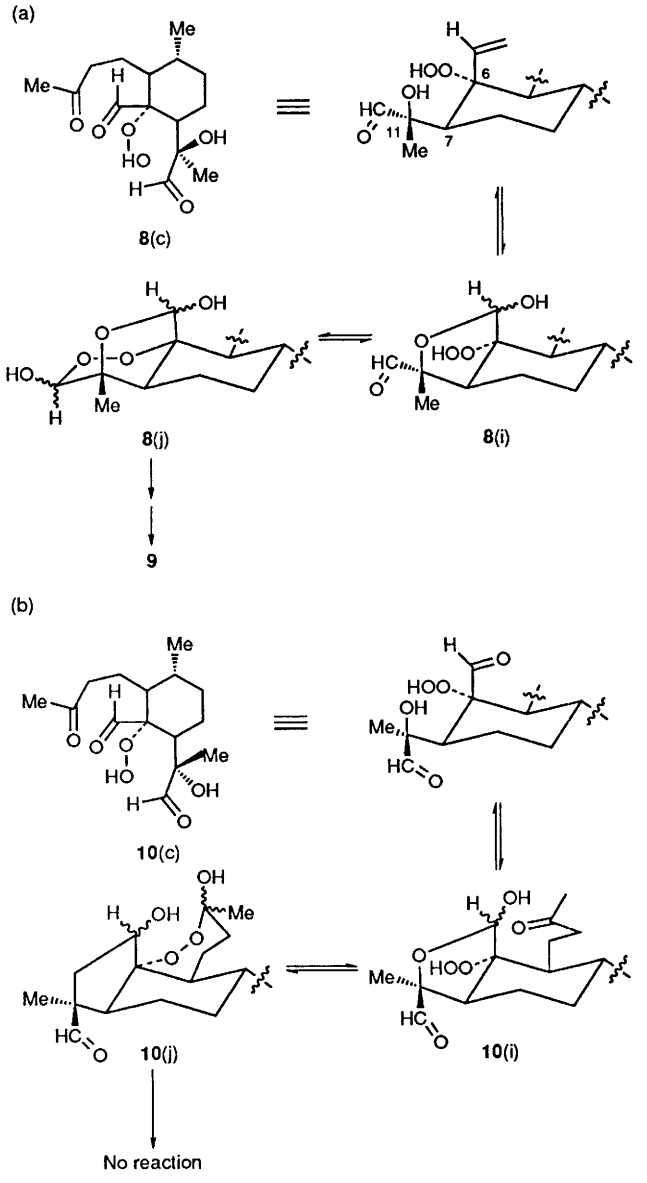


Fig. 2 Selected H–C couplings in the HMBC spectrum of compound **9**

spectrum suggested that the CH_2O group at C-12 was lost during the reaction. Removal of C-12 and the atoms attached to it requires the oxygen previously bonded to C-5 and C-12 to become bonded to C-11, forming a tetrahydrofuran. This deduction is consistent with the 40 ppm downfield shift of C-11 in the ^{13}C NMR spectrum of compound **9** compared with that in compound **8**. The loss of a second oxygen atom has been accounted for by transforming the 4,6-peroxide into a 4,6-oxide, a reaction reported by Posner and Oh for their synthetic 1,2,4-trioxane.[3] Support for the presence of a 4,6-oxide came from a comparison of the proton and carbon chemical shifts for 15-Me, C-4 and C-6 in several peroxides and the corresponding oxides (Table 2). The transformation of the peroxide into an oxide in these artemisinin derivatives is usually accompanied by a 2–4 ppm downfield shift of C-4 and C-6 in the ^{13}C NMR spectrum and a 0.1 ppm shift for 15-Me. The appropriate shifts in compound **9** are seen for C-4 and C-15 but the larger shift for C-6 may result from contraction of the D-ring as seen in Table 1. The stereochemistry for C-13 in compound **9** was initially assigned based on a comparison of its ^{13}C NMR chemical shift with that for the methyl groups in 11β-hydroxy- and 11α-hydroxydihydroartemisinin. The ^{13}C NMR chemical shifts of α-methyl groups vary from δ_C 28 to 30 whereas those for β-methyls vary between δ_C 22 and 23. Similar shifts were

Table 2 Selected NMR spectral data of the 4,6-peroxides and 4,6-oxides

Compound	Me at C-4 (δ_H)	C-4 (δ_C)	C-6 (δ_C)
1a	1.44	105.3	79.5
11	1.53	109.2	82.5
1b	1.43	104.1	80.3
6	1.54	108.1	82.4
Arteether **7**	1.42	104.0	81.2
Deoxyarteether	1.54	107.9	83.4



Scheme 2 Silica gel-catalysed reactions of compounds **8c** and **10c**. (a) A 1,3-*cis* relationship between 6-OOH and 11-CHO favours addition of the hydroperoxide to the aldehyde. (b) A 1,3-*trans* relationship between 6-OOH and 11-CHO does not permit addition of the hydroperoxide to the aldehyde.

observed by Lin *et al.* in the reaction products of compound **2** with MCPBA.[2a] The observed chemical shift (δ_C 22) for C-13 in compound **9** is closer to that of a β-methyl than to that for an α-methyl. The same stereochemical assignment can be deduced for compound **9** from data on the ^{1}H NMR chemical shift of the hydroxy group (δ 5.2) on C-11 compared with δ 2.3 for diol **8** and δ 4.6 in diol **10**. The 11α-configuration of the hydroxy group was verified by a 2D NOESY spectrum which showed a cross-peak between the hydroxy group and 7-H. The differences in the ^{13}C NMR shifts for C-5, C-6 and C-7 in compound **9** compared with diol **8** presumably are a consequence of the D-ring contracting from a six- to five-membered ring.

The HMBC spectrum was employed for unequivocal assignments of the ^{13}C NMR spectrum of compound **9** as seen in Fig. 2. The two-bond coupling of 5-H to C-6 identified C-6, and the three-bond coupling to C-11 requires the presence of an oxygen atom between C-5 and C-11. The stereochemical assignment of C-13 follows from that for the hydroxy group discussed above. The above spectra showed no features suggesting skeletal rearrangements.

Additional information on the reaction was obtained from unsuccessful attempts to catalyse the conversion of diol **8** into alcohol **9** by radical initiators. Heating of a chloroform solution of diol **8** in the presence of azoisobutyronitrile (AIBN) did not generate compound **9**. In an effort to effect analogous transformation in artemisinin derivatives, benzene solutions of compounds **1a**, **1b**, arteether **7** and compound **10** were refluxed in the presence of silica gel. Artemisinin **1a**, arteether **7** and compound **10** were recovered unchanged after being heated for up to 10 h. However, dihydroartemisinin **1b** yielded a less polar compound, **11**, in 29% yield. A molecular mass determination of compound **11** showed it had formed from substrate **1b** by loss of the elements of water. An examination of its ^{1}H and ^{13}C NMR spectra enabled us to identify compound **11** as deoxyartemisinin.[4] This compound has been isolated from *Artemisa annua*[5] and as a metabolite from the urine of malaria patients treated with artemisinin.[6]

A literature search for silica gel-catalysed reactions of artemisinin derivatives revealed a paper by El-Feraly *et al.*[7] describing the rearrangement of dihydroartemisitene **4** to the aldehyde **5**, whose structure was determined by X-ray crystallography. On the basis of our findings and El-Feraly's report, formation of compounds **9** and **11**, as well as of those compounds described in the literature, have been rationalized (Scheme 1). Under neutral and/or weakly acidic conditions the hemiacetal at C-12 is in equilibrium with structures (a), (b) and (c) as shown in Scheme 1. Compound **4** undergoes the reaction sequence **4**(a) to **4**(b) to **4**(c) followed by addition of the hydroperoxide to the C-12 aldehyde to form **4**(d), *e.g.* compound **5**. The reaction scheme thus accounts for El-Feraly's observations. Structures such as **5** have been proposed as intermediates in Baeyer–Villiger reactions.[8] An intermediate such as **8**(d) would be expected to undergo a Baeyer–Villiger reaction to form **8**(e), which then loses formic acid to form **8**(f) and then hemiacetal **9**. The failure of peroxide **5** to undergo a Baeyer–Villiger reaction might be due to the presence of the adjacent carbon–carbon double bond or to a difference in the reaction conditions used by El-Feraly *et al.*[7] To account for the observation that diol **10**(c) does not undergo an analogous transformation, we suggest that perhaps in the acetal **10**(i) (Scheme 2) the distance between the hydroperoxide and the aldehyde group is too large for formation of the key Baeyer–Villiger reaction intermediate.

The transformation of **1b** to lactone **11** was rationalized by assuming that the Baeyer–Villiger reaction proceeded by a hydride migration to form compound **1b**(g). Under the acidic conditions used, the hydroxy group of the hemiacetal **1b**(h) and the carboxy group react to form lactone **11**.

Posner and Oh suggested that the biotransformations of substrates **1a** or **1b** to 4,6-oxide **6** occur by radical reactions.[3] However, it is also possible to account for these transformations by non-radical processes as shown in Scheme 1 for the conversion of compound **1b** into lactone **11**. Transformation of dihydroartemisinin **1b** that is catalysed by weak acids on the surface of silica gel might also occur on cell surfaces, leading to loss of the peroxide group and as a consequence to the loss of the antimalarial activity of these drugs.

Conclusions.—The silica gel-catalysed reactions observed for two artemisinin derivatives (**1b** and **8**) demonstrate that the 1,2,4-trioxane system opens and undergoes a series of reactions under very mild acidic conditions on silica gel surfaces. The configuration of the hydroxy group at C-11 controls the conversion of peroxide **8** into oxide **9**.

Experimental

Unless otherwise stated, the m.p.s were determined using a Reichert hot-stage apparatus and are uncorrected. Sodium borohydride, phosphorus pentaoxide, potassium fluoride and MCPBA (80–90%) were purchased from Aldrich and used without purification. All other reagents were ACS grade or the highest quality material available. Silica gel TLC plates and TLC-grade silica gel (cat. no. 10050) were from Analtech, Newark, DE 19713. Dichloromethane was dried over P_2O_5 and distilled. ^1H NMR and ^{13}C NMR spectra were run on a VXR-500 or Gemini 300 spectrometer using Me_4Si as a standard in $CDCl_3$. *J*-Values are given in Hz. FT-IR spectra were measured on a Bio-Rad FTS-45 spectrometer, and CI-MS were determined on a Finnagan 4600 Mass Spectrometer. Microanalyses were performed by Galbraith Laboratory, Inc., Tennessee. Optical solutions (reported in units of 10^{-1} deg cm^2 g^{-1}) were measured on a Perkin Elmer 241 MC polarimeter.

MCPBA·2KF Complex.—A mixture of finely grounded potassium fluoride (2.2 g, 0.038 mol) and MCPBA (3.2 g, 0.016 mol) were suspended in CH_2Cl_2 (110 cm^3), and stirred at room temperature for 7 h. The precipitate (MCPBA·2KF) was filtered off, washed with dry CH_2Cl_2, and stored at −20 °C until used.

Epoxide **3** *and* 11α-*Hydroxydihydroartemisinin* **10**.—The MCPBA·2KF complex (3.0 g) and compound **2**[2b] (600 mg, 2.25 mmol) were suspended in dry CH_2Cl_2 (70 cm^3). The suspension was stirred at 4 °C for 66 h, then was filtered through a layer of anhydrous $NaHCO_3$. The filtrate was evaporated to dryness (570 mg) and epoxide **3** was purified by flash chromatography on a Buchner funnel (300 cm^3) tightly packed with TLC-grade silica gel with hexane–ethyl acetate (5:1) as eluent. Compound **3** [(460 mg, 70%), m.p. 117–119 °C (lit.,[2b] 117–120 °C)] was eluted first, and further washing with hexane–acetone (7:3) afforded compound **10** (115 mg, 17%), m.p. 145–150 °C (Found: C, 60.25; H, 8.1. $C_{15}H_{24}O_6$ requires C, 59.98; H, 8.05%); $[\alpha]_D$ +82 (*c* 0.22, $CHCl_3$); CI-MS (NH_3): 318 ($M + NH_4^+$, 100%) and 300 ($M + NH_4^+ - H_2O$, 75); δ_H **10a**: 5.40 (1 H, s, 5-H), 4.76 (1 H, s, 12-H), 4.36 and 3.86 (2 H, s, D_2O-exchangeable, 11- and 12-OH), 2.4–0.9 (11 H, overlapping carbon-skeleton protons), 1.46 (3 H, s, 15-H), 1.12 (3 H, s, 13-H) and 0.97 (3 H, d, *J* 5.8, 14-H_3); **10b**: 5.69 (1 H, s, 5-H), 5.12 (1 H, s, 12-H), 4.68 and 3.0 (2 H, s, D_2O-exchangeable, 11- and 12-OH), 2.4–0.9 (11 H, overlapping carbon-skeleton protons), 1.46 (3 H, s, 15-H), 1.21 (3 H, s, 13-H) and 0.96 (3 H, d, *J* 5.8, 14-H); δ_C (Table 1).

11β-*Hydroxy*-11-*epidihydroartemisinin* **8**.—To a solution of epoxide **3** (500 mg, 1.77 mmol) in acetone (120 cm^3)–water (40 cm^3) was added 1 mol dm^{-3} H_2SO_4 (0.1 cm^3). The solution was stirred at room temperature for 5 min and was then extracted with CH_2Cl_2. The extract was washed with 1% aq. $NaHCO_3$, dried over Na_2SO_4, and evaporated to dryness. Flash chromatography on silica gel (TLC grade) with hexane–acetone (3:1) as eluent yielded diol **8** (430 mg, 80%), m.p. 133–135 °C (Found: C, 60.1; H, 8.1. $C_{15}H_{24}O_6$ requires C, 59.98; H, 8.05%); $[\alpha]_D$ +70 (*c* 0.30, $CHCl_3$); CI-MS (NH_3): 318 ($M + NH_4^+$, 40%) and 300 ($M + NH_4^+ - H_2O$, 60); δ_H **8a**: 5.37 (1 H, s, 5-H), 4.95 (1 H, s, 12-H), 3.35 and 2.30 (2 H, s, D_2O-exchangeable, 12- and 11-OH), 2.4–0.9 (11 H, overlapping carbon-skeleton protons), 1.53 (3 H, s, 13-H), 1.40 (3 H, s, 15-H) and 0.94 (3 H, d, *J* 5.8, 14-H); **8b**: 5.51 (1 H, s, 5-H), 5.26 (1 H, s, 12-H), 3.80 and 2.30 (2 H, s, D_2O-exchangeable, 12- and 11-OH), 2.4–0.9 (11 H, overlapping carbon-skeleton protons), 1.45 (3 H, s, 13-H), 1.40 (3 H, s, 15-H) and 0.94 (3 H, d, *J* 5.8, 14-H); δ_C (Table 1).

Preparation of Oxide **9**.—To a solution of diol **8** (50.0 mg, 0.17 mmol) in benzene (5.0 cm^3) was added TLC-grade silica gel (500 mg). The mixture was reluxed for 10 min, then filtered, and the silica gel was washed with acetone. The filtrate and washings were combined and evaporated to dryness. Purification on a preparative TLC (PLC) plate with hexane–ethyl acetate (7:3) as developer gave compound **9** (20.0 mg, 70% based on diol **8** consumed in the reaction) as an oil (Found: M^+, 254.1525. $C_{14}H_{22}O_4$ requires M, 254.1517); $[\alpha]_D$ −65 (*c* 0.85, $CHCl_3$); δ_H 5.65 (1 H, s, 5-H), 5.20 (1 H, s, D_2O-exchangeable, 11-OH), 2.3–1.0 (11 H, overlapping carbon-skeleton protons), 1.58 (3 H, s, 15-H), 1.35 (3 H, s, 13-H) and 0.96 (3 H, d, *J* 5.5, 14-H); δ_C (Table 1).

Preparation of Lactone **11** *from Hemiacetal* **1b**.—To a solution of compound **1b** (50.0 mg, 0.18 mmol) in benzene (5.0 cm^3) was added TLC-grade silica gel (500 mg). The mixture was refluxed for 6 h, then filtered, and the silica gel was washed with acetone–methanol (5:1). The filtrate and washings were combined and evaporated to dryness. Purification on a PLC plate with hexane–ethyl acetate (7:3) as developer afforded lactone **11** (12.0 mg, 29% based on the consumed starting material) and recovered compound **1b** (8.0 mg). Compound **11**: m.p. 110–112 °C (lit.,[1e] 111–113 °C); δ_H 5.70 (1 H, s, 5-H), 3.20 (1 H, m, 11-H), 1.53 (3 H, s, 15-H), 1.20 (3 H, d, *J* 7.2, 13-H) and 0.94 (3 H, d, *J* 5.6, 14-H).

Note added in proof: J. K. Baker, J. D. McChesney and H. T. Chi (*Pharm. Res.*, 1993, **10**, 662) have reported the isolation of **1d** (Scheme 1) by treating arteether with HCl (2.5 mol dm^{-1}) in ethanol–water (1:1).

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