



A Rearranged Hydroperoxide from the Reduction of Artemisinin

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Abstract: Lithium aluminium hydride reduction of artemisinin produces two unexpected rearrangement products in addition to those reported previously. The structure of the major rearrangement product, a tertiary hydroperoxide, was determined by 2D-NMR and chemical reactions. A mechanism is proposed for this rearrangement, based on the observation that dihydroartemisinin can also be converted into the same hydroperoxide in alkaline solution. © 1997 Elsevier Science Ltd.

Artemisinin (**1**), the active principle from the Chinese anti-malarial plant *Artemisia annua*, was first isolated in 1972.¹ X-ray crystallographic analysis demonstrated that artemisinin incorporated a unique 1,2,4-trioxane system and subsequent research has established that this is the principal structural requirement for the biological activity of **1**.² It is currently believed that artemisinin exerts its biological activity by radical formation following cleavage of the endoperoxide linkage of the 1,2,4-trioxane ring by iron in haem groups of red blood cells, associated with the malarial parasites.^{3,4}

Over the past twenty years there has been much interest in the chemistry of **1**, (extensively reviewed in references 5-7) partly in respect of its unusual structure and also in the search for analogues which might serve as clinical alternatives. Sodium borohydride reduction of artemisinin, for example, yields dihydroartemisinin (**2**) (as a mixture of diastereoisomers); the ethyl ether of **2** is many times more water-soluble than **1** and is sold commercially as "arteether".⁸ Artemisinin and its derivatives have been shown to undergo a variety of unusual rearrangement reactions under thermal⁹⁻¹², basic¹³⁻¹⁵ and acidic conditions.¹⁶⁻¹⁹ In this study, we describe another unexpected reaction of artemisinin associated with lithium aluminium hydride reduction, in which the 1,2,4-trioxane system rearranges to a tertiary hydroperoxide at the junction of 5- and 6-membered rings.

RESULTS AND DISCUSSION

LiAlH₄ reduction of **1** has been reported to result in complete reduction to compound **6** and partial reduction to the cyclic hemiacetal **5**.²⁰ As part of our investigation of the chemistry of artemisinin, we have repeated this reaction and isolated two novel compounds (**3** and **4**), in addition to those previously reported.

The planar structure of the major product **3** from the reduction was deduced largely from analysis of 1D- (¹H, ¹³C/DEPT) and 2D-NMR spectra (HSQC, HMBC and ¹H-¹H COSY) which established the skeleton shown in Figure 1, consisting of fused 5 and 6-membered rings and provided ¹³C and ¹H NMR chemical shift assignments for all positions (Table 1). Additional correlations between position 5 and

position 12 implied the existence of a heterocyclic ring linking together these centres. From ^{13}C chemical shift values, C-4 was obviously a carbonyl, whilst the 5-, 6- and 12 positions apparently bore oxygen substituents. Two broad proton resonances at δ 4.50 and 9.85 ppm disappeared from the ^1H NMR spectrum on shaking with D_2O ; these chemical shift values are consistent with the presence of hydroxy and hydroperoxy²¹ groups, respectively. Analysis of the ^{13}C spectrum following D_2O exchange showed a large upfield shift at C-12 ($\Delta = -0.12$ ppm; see Table 1), a moderate upfield shift at C-6 and little change for the resonance at C-5, consistent with location of the hydroxy group at position 12 and an ether linkage at position 5 (carbon atoms attached directly to OH groups are expected to move upfield by 0.1-0.2 ppm as a result of secondary isotope effects following exchange of H by D; adjacent carbon atoms show smaller shifts²²).

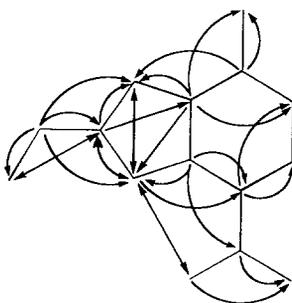
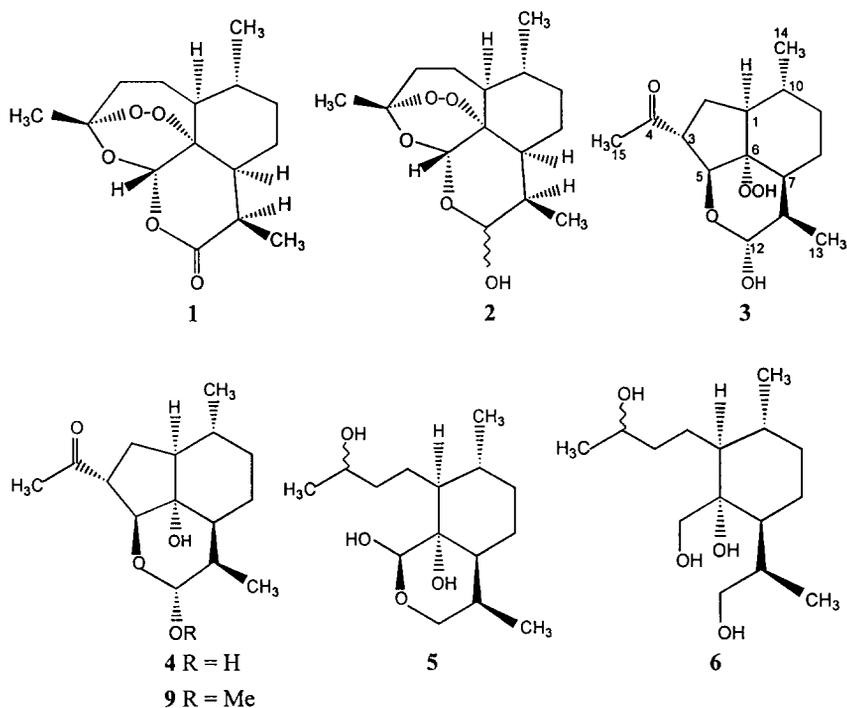


Figure 1. Skeleton of **3** as established by HSQC/HMBC. 2 and 3-bond correlations from ^{13}C to ^1H indicated by single-headed arrows; double-headed arrows indicate reciprocal correlations (identical pattern of correlations observed for **4** and **9**)

Table 1. NMR data for 3, 4 and 9

Compound	Multiplicity ^a	3			4			9	
		$\delta^{13}\text{C}$	$\Delta^{13}\text{C}(\text{D}_2\text{O})^{\text{b}}$	$\delta^1\text{H}$	$\delta^{13}\text{C}$	$\Delta^{13}\text{C}(\text{D}_2\text{O})^{\text{b}}$	$\delta^1\text{H}$	$\delta^{13}\text{C}$	$\delta^1\text{H}$
1	CH	47.2	+0.05	2.10	55.0	-0.07	1.52	55.6	1.47
2	CH ₂ α β	28.4	+0.05	2.07	29.0	+0.03	2.12	28.4	2.17
				1.92			1.92		1.91
3	CH	57.0	+0.02	2.95	57.4	+0.01	2.96	57.3	2.95
4	C	210.0	+0.21		209.9	+0.19		208.8	
5	CH	75.5	-0.02	3.99	80.1	-0.12	4.11	80.3	3.95
6	C	93.3	-0.07		81.6	-0.13		81.5	
7	CH	37.7	+0.02	2.15	45.7	-0.07	1.62	45.8	1.57
8	CH ₂ α β	23.3	-0.01	1.82	23.9	-0.02	1.76	24.1	1.78
				1.24			1.22		1.21
9	CH ₂ α β	32.0	0.00	0.97	32.6	-0.01	0.95	32.6	0.94
				1.60			1.65		1.66
10	CH	32.8	0.00	1.30	32.9	-0.01	1.25	33.0	1.26
11	CH	34.3	-0.07	2.18	35.1	-0.09	2.08	33.8	2.07
12	CH	98.1	-0.12	4.82	98.5	-0.12	4.85	105.6	4.36
13	CH ₃	14.6	-0.01	1.07	15.0	-0.01	1.10	14.9	1.06
14	CH ₃	20.9	0.00	0.97	21.0	0.00	0.93	21.0	0.94
15	CH ₃	28.5	+0.03	2.22	29.3	+0.02	2.24	29.0	2.25
-OMe								55.0	3.41

^a Multiplicity established by DEPT is the same for all carbon positions in compounds 3, 4 and 9.

^b Negative values (ppm) denote an upfield shift in ^{13}C resonance following D_2O shake.

This interpretation was supported by the results of a D_2O shake experiment with compound 4, the 6-hydroxy analogue of 3, which gave comparably large upfield shifts in the ^{13}C spectrum for both C-6 (-0.13 ppm) and C-12 (-0.12 ppm) following deuterium exchange (Table 1). Compound 4 was isolated as a minor product from the reduction reaction: its structure was determined by 2D-NMR in the same manner as for 3, and the pattern of correlations observed in HMBC was essentially identical to that for 3 (see Figure 1). Complete ^{13}C and ^1H chemical shift assignments for the two compounds (Table 1), established by 2D-NMR techniques, showed strong similarities with the exception of C-6 which was more than 10 ppm downfield in compound 3 as compared with 4. This is consistent with the presence of a hydroperoxide group²³ at C-6 in 3 as opposed to a hydroxide group in 4. Further evidence for the presence of a hydroperoxide group in 3 was obtained by chemical means, since reduction of 3 with triphenylphosphine lead to clean conversion into 4.

Compounds 3 and 4 possessed the same relative stereochemistry, as both gave an identical pattern of correlations in NOESY spectra (Figure 2). The absolute stereochemistry of 4 was confirmed by X-ray crystallography (Figure 3). Since compound 3 could only be obtained as an oil its absolute stereochemistry is assumed to be the same as that found for 4. Neither 3 nor 4 gave molecular ions in HREIMS but both produced daughter ions corresponding to a loss of water (molecular formulae $\text{C}_{15}\text{H}_{22}\text{O}_4$ and $\text{C}_{15}\text{H}_{22}\text{O}_3$ respectively).

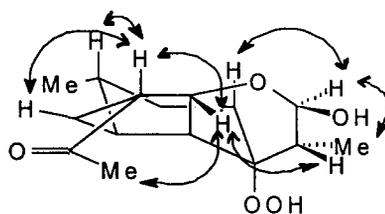


Figure 2. Selected NOESY correlations between protons of **3** indicated by double-headed arrows (identical pattern of correlations observed for **4** and **9**).

Compounds **5** and **6** were also isolated from the reaction mixture (as 2:1 and 1:1 mixtures of diastereoisomers, respectively). Their structures were rigorously established by 2D-NMR as previously (Table 2).

Table 2 ^{13}C and ^1H NMR data for **5-6**

Atom	$\delta^{13}\text{C}$				$\delta^1\text{H}$			
	5a*	5b*	6a+	6b+	5a*	5b*	6a+	6b+
1	50.6	52.0	53.7	52.5	1.05	1.10	1.15	1.08
2	20.3	20.8	23.5	25.6	1.80 1.40	1.60 1.40	1.92	1.92
3	35.0	35.5	39.1	40.6	1.70 0.90	1.80 1.10	1.40 1.50	1.60 1.35
4	65.9	65.6	64.9	69.6	3.90	3.90	3.92	3.77
5	95.5	93.8	59.6	59.5	5.05	5.01	3.85 3.34	3.85 3.34
6	73.6	74.0	75.6	75.8				
7	45.9	48.1	53.3	53.4	1.45	1.60	1.35	1.35
8	38.6	39.5	35.7	35.7	1.55 1.55	1.35 1.35	1.20 1.00	1.20 1.00
9	22.1	22.8	22.8	22.9	1.70 1.40	2.20 1.30	1.65 1.45	1.65 1.45
10	34.7	35.2	35.5	35.3	2.00	1.50	1.70	1.70
11	28.9	28.7	31.6	31.6	2.35	2.40	2.35	2.35
12	61.0	67.4	65.0	65.0	3.75 3.30	3.62 3.42	3.54 3.40	3.54 3.40
13	14.0	13.3	20.5	20.5	0.78	0.76	0.88	0.88
14	21.4	20.9	20.8	19.8	0.89	0.94	0.88	0.88
15	22.8	22.9	22.5	23.0	1.17	1.17	1.17	1.15

* 5a/5b approximate ratio 2:1

+ 6a/6b approximate ratio 1:1. Assignments between two isomers interchangeable

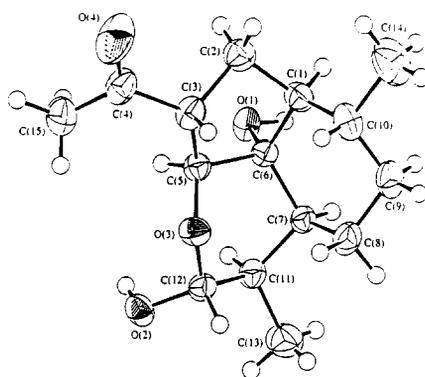


Figure 3. ORTEP diagram of compound 4

The course of the LiAlH_4 reduction of **1** can be rationalized on the assumption that initial reduction occurs at the lactone functional group, producing the hemi-acetal anion **7**, which can then cause the 1,2,4 trioxane ring to "unzip" as shown in Figure 4. The resulting ring-opened peroxide anion **8** may then act as an internal base to induce enolization of the carbonyl. This enolate then undergoes aldol reaction with the 5-aldehyde group to produce a new 5-membered carbocyclic ring which leads directly to compound **3** upon hemi-acetal formation (further reduction at the hydroperoxide group generates **4**). Alternatively, further reduction of intermediate **8** at the 4, 5, 6 and 12 centers leads to the partially reduced product **5** and fully reduced product **6**.

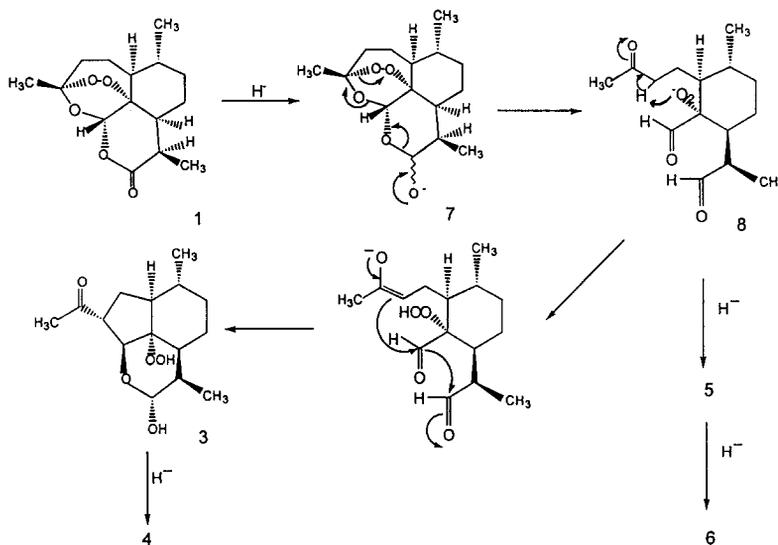


Figure 4. Formation of compounds **3-6** from **1** by initial reduction at the lactone.

This mechanism for the formation of **3** and **4** received support from the observation that alkaline treatment of **2** (obtained as a mixture of diastereoisomers from **1** by NaBH_4 reduction) leads exclusively to **3** in high yield. Compound **2** would be expected to form the anion **7**, postulated in the lactonic reduction of artemisinin (Figure 4), under alkaline conditions. The anion **7** then undergoes "unzipping" and aldolization

reactions to yield **only 3** in the absence of reducing agent. Prolonged base treatment of **2** resulted in conversion to the methylated cyclic hemi-acetal **9**. The methoxy substituent in **9** was established as being α by NOESY (other NOESY correlations were as observed for **3** and **4**).

EXPERIMENTAL

General methods.

Chemical shifts are expressed in ppm (δ) relative to TMS as int. standard. All NMR experiments were run on Bruker DPX 300 or DRX 500 instruments in CDCl_3 . Two dimensional spectra were recorded with 1024 data points in F_2 and 256 data points in F_1 . HREIMS were recorded at 70 e.v. on a Finnigan-MAT 95 MS spectrometer. IR spectra were recorded in solution on a BIO-RAD FT S-7 IR spectrometer. TLC plates were developed using *p*-anisaldehyde. Column chromatography was performed using silica gel 60-200 μm (Merck). HPLC separations were performed using a PREP-SIL 20 mm x 25 cm column, flow rate 8 ml/min. Artemisinin (1Kg; 100.98%, batch no. 950503) was obtained from Kunming Pharmaceutical Factory, Qigongli West Suburb, PRC.

Reduction of artemisinin (1) by LiAlH_4 . A solution of artemisinin in anhydrous ether (6.2 g/17 ml) was added to a stirred solution of ethereal lithium aluminium hydride (0.85g/70ml) over 2 hours with stirring²⁴. After adding additional anhydrous ether (50 ml), the reaction mixture was heated under reflux for a further 3 hrs. Saturated Na_2SO_4 solution (5 ml) was added to the ice-cooled reaction mixture to hydrolyse excess hydride. Following stirring for a further 2 hours, a solid precipitate formed which was separated by filtration and washed several times with ether. The combined organic extracts were dried and solvent removed to obtain a crude oil (5.84 g). Gradient elution column chromatography of this oil (4.4g) yielded **1** (0.33g), **3** (R_f 0.4 70% EtOAc/*n*-hexane; 0.45g), **4** (R_f 0.4 85% EtOAc/*n*-hexane, 0.12g), **5** (R_f 0.3 100% EtOAc; 0.02g) and **6** (R_f 0.4 10% MeOH/EtOAc; 0.52g). **Compound 3**. Colourless oil. $[\alpha]_D = -75.1^\circ$ (c 3.0 CHCl_3). HREIMS m/z (% intensity): 266.1518 (8) $[\text{M}-\text{H}_2\text{O}]$ ($\Delta = 0.0$ mmu for $\text{C}_{15}\text{H}_{22}\text{O}_4$), 248 (26), 232 (40), 208 (100), 190 (41), 161 (23), 149 (32). IR (CCl_4) $\nu \text{ cm}^{-1}$: 3591, 3412 (br), 3003, 2930, 1707. $^1\text{H NMR } \delta$ (ppm): 9.85 (1H, br s, exch. D_2O), 4.82 (1H, d, $J=7.5$ Hz), 4.50 (1H, br s, exch. D_2O), 3.99 (1H, d, $J=6.3$ Hz), 2.95 (1H, br m), 2.22 (3H, s), 1.07 (3H, d, $J=6.6$ Hz), 0.97 (3H, d, $J=6.2$ Hz). **Compound 4**. Crystals. Mp 171-173°C. $[\alpha]_D = -79.0^\circ$ (c 1.86 CHCl_3). HREIMS m/z (% intensity): $[\text{M}-\text{H}_2\text{O}]$ ($\Delta = 0.3$ mmu for $\text{C}_{15}\text{H}_{22}\text{O}_3$) (47), 232 (100), 207 (48), 161 (43), 149 (33). IR (CHCl_3) $\nu \text{ cm}^{-1}$: 3287 (br), 2930, 2878, 2833, 1707. $^1\text{H NMR } \delta$ (ppm): 4.85 (1H, d, $J=6.6$ Hz), 4.11 (1H, d, $J=6.2$ Hz), 3.47 (1H, br.s, exch. D_2O), 2.96 (1H, ddd, $J=7.0, 6.2, 6.2$), 2.24 (3H, s), 1.70 (1H, brs, exch. D_2O), 1.10 (3H, d, $J=7.0$ Hz), 0.93 (3H, d $J=6.5$ Hz). **Compound 5a/5b** (inseparable mixture of diastereoisomers, 2:1 ratio). Oil. HREIMS m/z (% intensity) 272.1986 (0.2) $[\text{M}^+]$ ($\Delta = 0.1$ mmu for $\text{C}_{15}\text{H}_{28}\text{O}_4$), 254 (10), 225 (11), 211 (27), 208 (25), 193 (100), 165 (67), 151 (61). IR (CCl_4) $\nu \text{ cm}^{-1}$: 3367 (br), 2931, 2876. $^1\text{H NMR } \delta$ (ppm) Major isomer (**5a**) 5.05 (1H, d, $J=4.0$ Hz), 4.68 (1H, d, $J=4.0$ Hz), 3.90 (1H, br m), 3.75 (1H, t, $J=11.5$ Hz), 3.30 (1H, dd, $J=11.5, 4.5$ Hz), 1.17 (3H, d, $J=6.2$ Hz), 0.89 (3H, d, $J=6.4$ Hz), 0.78 (3H, d, $J=7.0$ Hz). Minor isomer (**5b**) 5.01 (1H, d, $J=7.2$ Hz), 4.93 (1H, d, $J=7.2$ Hz), 3.90

(1H, br m); 3.62 (1H, dd, $J=11.5, 5.0$ Hz), 3.42 (1H, t, $J=11.5$ Hz), 1.17 (3H, d, $J=6.2$ Hz), 0.94 (3H, d, $J=6.7$ Hz), 0.76 (3H, d, $J=7.1$ Hz). **Compound 6a/6b**. Oil. HREIMS m/z (% intensity) 274.2145 (0.8) [M^+] ($\Delta = -0.1$ mmu for $C_{15}H_{30}O_4$), 256 (5); 238 (5); 225 (100); 207 (52); 165 (29); 149 (30); 109 (15). IR (CCl_4) ν cm^{-1} 3323 (br), 2966, 2927.

Reduction of artemisinin (1) by $NaBH_4$. To a stirred solution of artemisinin (2.4g) in methanol (120 ml) cooled in an ice-salt bath was added $NaBH_4$ (2.4 g) gradually over a period of 100 mins. Stirring was continued for a further 75 mins before the mixture was neutralized by addition of acetic acid, maintaining the temperature between 0-5°C. The solution was concentrated by distilling off solvent, diluted with cold water (75 ml) and stirred for 15 mins at room temperature. A white precipitate was collected and washed with H_2O -MeOH (2:1, 0-5°C). Storage of the filtrate for 14 hours at 4°C resulted in a further small amount of precipitate, which was collected and washed as before. After drying ($MgSO_4$) and evaporation of solvent, compound **2** (1.6 g) was obtained⁸ as a 1:1 mixture of diastereoisomers. Solid. Mp 181-183°C. $[\alpha]_D = 137.7^\circ$ (c 3.3 $CHCl_3$). HREIMS m/z (% intensity): 266.1519 [$M-H_2O$] ($\Delta = -0.1$ for $C_{15}H_{22}O_4$) (5), 252 (45), 234 (50), 195 (50), 194 (100). IR ($CHCl_3$) ν cm^{-1} : 3427 br, 2930, 2876. 1H NMR δ (ppm): 5.39 (1H, s)/5.61 (1H, s), 4.75 (1H, t, $J=8.9$ Hz)/5.30 (1H, t, $J = 3.3$ Hz), 3.17 (1H, m)/2.86 (1H, m), 2.38 (1H, td, $J=15.9, 3.9$ Hz), 1.43 (3H, s)/1.42 (3H, s), 0.94-0.96 (6H, several doublets). ^{13}C NMR/DEPT δ (ppm): 104.4/104.1 C; 96.4/94.6 CH; 91.2/87.7 CH; 80.4/81.1 C; 51.5/52.5 CH; 45.4/44.3 CH; 37.4/37.5 CH; 36.4/36.2 CH_2 ; 34.8/30.8 CH; 34.2/34.7 CH_2 ; 26.0/25.95 CH_3 ; 24.72/24.68 CH_2 ; 24.57/22.13 CH_2 ; 20.4/20.3 CH_3 ; 13.2/12.7 CH_3

Conversion of 2 to 3. Compound **2** (0.50g) was added to aqueous potassium hydroxide (10% w/v, 10 ml) and a small amount of methanol included in the mixture to enhance solubility. The reaction mixture was stirred for 15 mins at room temperature and then neutralized with dilute HCl. Immediate work-up (extraction into ether, drying over $MgSO_4$ and removal of solvent) yielded **3** (0.40g), without need for further purification.

Conversion of 2 to 9. Compound **2** (0.10g) was added to a methanolic potassium hydroxide solution (10% w/v, 2ml) and stirred overnight. The mixture was then acidified with dilute HCl, extracted with diethyl ether (20 ml) and washed with water. A pale yellow gum was collected following drying and removal of solvent. Compound **9** (10 mg) was isolated by HPLC, using a mixture of *n*-hexane and ethyl acetate (1:1 v/v) as eluent. Solid. Mp 115-116°C $[\alpha]_D = -97.5^\circ$ (c 1.85 $CHCl_3$). HREIMS m/z (% intensity): 264.1725 (M^+-H_2O) ($\Delta = 0.0$ for $C_{16}H_{24}O_3$), 232 (100), 221 (20), 189 (40), 124 (35). IR ($CHCl_3$) ν cm^{-1} : 3479 (br), 2936, 2880, 1709. 1H NMR δ (ppm): 4.36 (1H, d, $J=7.6$ Hz), 3.95 (1H, d, $J=6.3$ Hz), 3.41 (3H, s), 2.95 (1H, ddd, $J=13.6, 6.4, 6.4$ Hz), 2.25 (3H, s), 1.06 (3H, d, $J= 7.1$ Hz), 0.94 (3H, d, $J=6.4$ Hz).

Reduction of 3 to 4. A solution of **3** (53.3 mg) in CH_3OH (2.5 ml) was treated with triphenylphosphine (52.5 mg). After stirring for 2 hr at room temperature, the solution was evaporated to dryness and the residue was separated by column chromatography to yield **4** (20mg).²⁵

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