Pergamon

Tetrahedron, Vol. 53, No. 22, pp. 7493-7500, 1997

0040-4020/97 $17.00 + 0.00

PII: S0040-4020(97)00444-4

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 in1972.[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 thermal[9-12], basic[13-15] 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

$LiAlH_4$ reduction of **1** has been reported to result in complete reduction to compound **6** and partial reduction to the cyclic hemi-acetal **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, ^{13}C/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 ^{13}C and ^1H NMR chemical shift assignments for all positions (Table 1). Additional correlations between position 5 and

position 12 implied the existence of a heterocylic 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 ^{1}H NMR spectrum on shaking with D_2O; these chemical shift values are consistent with the presence of hydroxy and hydroperoxy[21] groups, respectively. Analysis of the ^{13}C spectrum following D_2O exchange showed a large upfield shift at C-12 (Δ = -0.12 ppm; see Table 1), a moderate upfield shift at C-6 and little change for the resonance at C-5, consitent 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 shifts[22]).

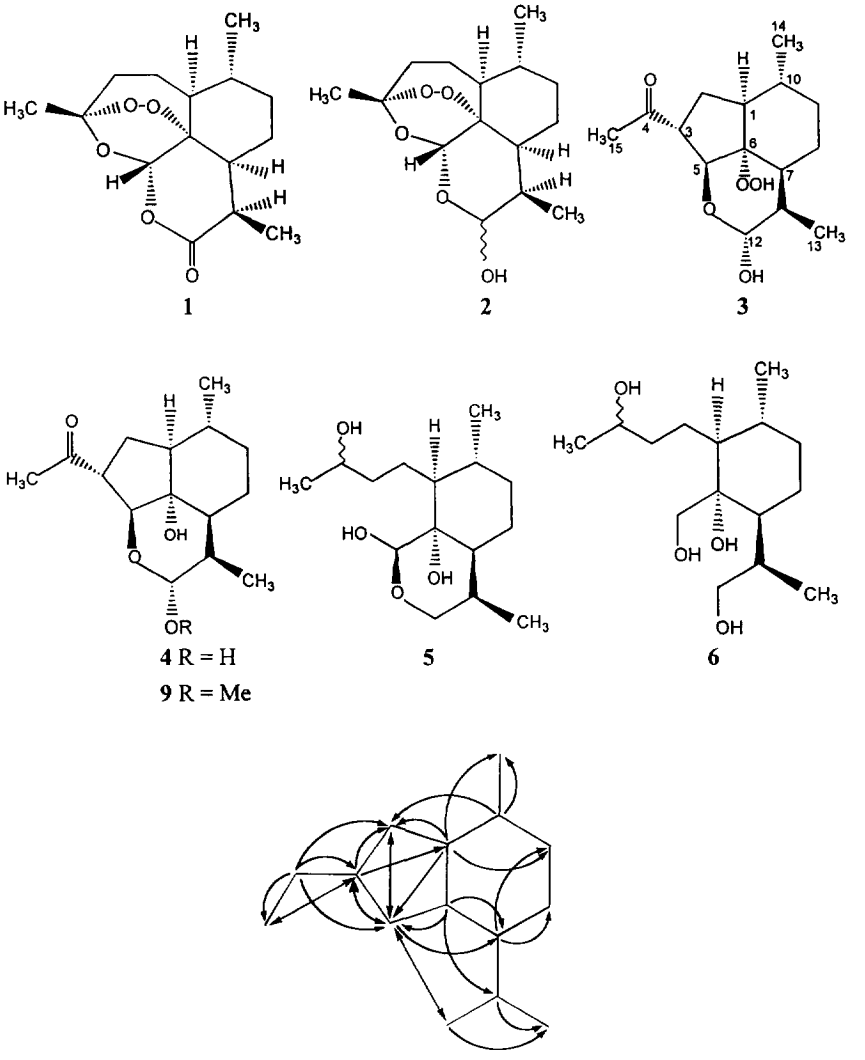

Figure 1. Skeleton of **3** as established by HSQC/HMBC. 2 and 3-bond correlations from ^{13}C to ^{1}H indicated by single-headed arrows; double-headed arrows indicate reciprocal correlations (identical pattern of correlations observed for **4** and **9**)

Table 1. NMR data for **3**, **4** and **9**

Compound		3			4			9	
	Multiplicity[a]	$\delta^{13}C$	$\Delta^{13}C$ (D_2O)[b]	$\delta^{1}H$	$\delta^{13}C$	$\Delta^{13}C$ (D_2O)[b]	$\delta^{1}H$	$\delta^{13}C$	$\delta^{1}H$
1	CH	47.2	+ 0.05	2.10	55.0	-0.07	1.52	55.6	1.47
2	CH_2 α	28.4	+0.05	2.07	29.0	+0.03	2.12	28.4	2.17
	β			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	23.3	-0.01	1.82	23.9	-0.02	1.76	24.1	1.78
	β			1.24			1.22		1.21
9	CH_2 α	32.0	0.00	0.97	32.6	-0.01	0.95	32.6	0.94
	β			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 Multuplicity 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 reults 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 essentialy identical to that for **3** (see Figure 1). Complete ^{13}C and ^{1}H 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 group[23] 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 $C_{15}H_{22}O_4$ and $C_{15}H_{22}O_3$ respectively).

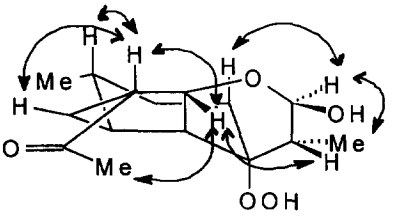

Figure 2. Selected NOESY correlations between protons of **3** indicated by double-headed arrows (identical pattern of correlations observed for **4** and **9**).

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 ^{1}H NMR data for **5-6**

	δ ^{13}C				δ ^{1}H			
Atom	**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 betweem two isomers interchangeable

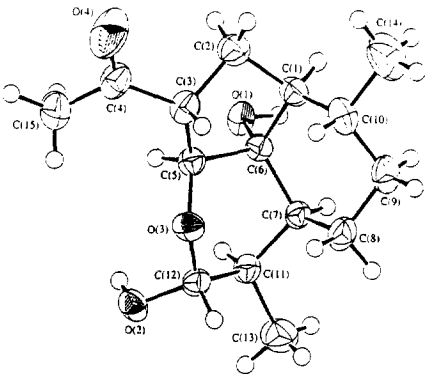

Figure 3. ORTEP diagram of compound **4**

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-openned 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**.

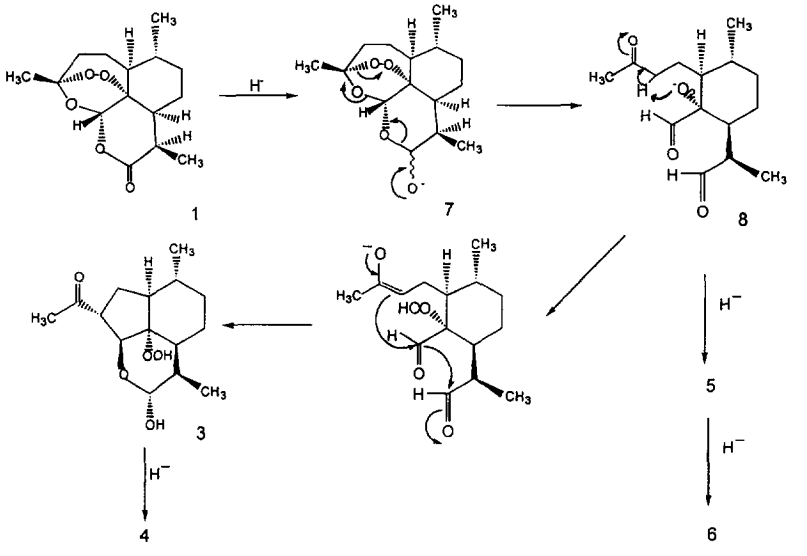

Figure 4. Formation of compounds **3-6** from **1** by initial reduction at the lactone.

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 stirring[24]. 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° (c 3.0 $CHCl_3$). HREIMS *m/z* (% intensity): 266.1518 (8) [M-H_2O] (Δ= 0.0 mmu for $C_{15}H_{22}O_4$), 248 (26), 232 (40), 208 (100), 190 (41), 161 (23), 149 (32). IR (CCl_4) ν cm^{-1}: 3591, 3412 (br), 3003, 2930, 1707. ^1H NMR δ (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° (c 1.86 $CHCl_3$). HREIMS 250.1566 *m/z* (% intensity): [M-H_2O] (Δ = 0.3 mmu for $C_{15}H_{22}O_3$) (47), 232 (100), 207 (48), 161 (43), 149 (33). IR ($CHCl_3$) ν cm^{-1}: 3287 (br), 2930, 2878, 2833, 1707. ^1H NMR δ (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** (inseperable mixture of diastereoisomers, 2:1 ratio). Oil. HREIMS *m/z* (% intensity) 272.1986 (0.2) [M^+] (Δ = 0.1 mmu for $C_{15}H_{28}O_4$), 254 (10), 225 (11), 211 (27), 208 (25), 193 (100), 165 (67), 151 (61). IR (CCl_4) ν cm^{-1}: 3367 (br), 2931, 2876. ^1H NMR δ (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^+] (Δ = -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 concentreated 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 obtained[8] as a 1:1 mixture of diastereoisomers. Solid. Mp 181-183°C. $[\alpha]_D$ = 137.7° (c 3.3 $CHCl_3$). HREIMS *m/z* (% intensity): 266.1519 [M-H_2O] (Δ = -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° (c 1.85 $CHCl_3$). HREIMS *m/z* (% intensity): 264.1725 (M^+-H_2O) (Δ = 0.0 for $C_{16}H_{24}O_3$], 232 (100), 221 (20), 189 (40), 124 (35). IR ($CHCl_3$) υ 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]

Acknowledgements: We would like to thank the CRCG for funding this research.

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(*Received in Japan* 18 *March* 1997; *accepted* 14 *April* 1997)