Synthesis and Antimalarial Activities of 12β-Allyldeoxoartemisinin and Its Derivatives

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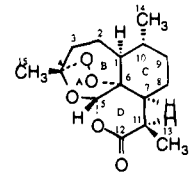
Received September 2, 1994[⊗]

Synthesis of 12β-allyldeoxoartemisinin from dihydroartemisinin and subsequent transformations to other 12β-alkyldeoxoartemisinins are described. All compounds were tested *in vitro* versus two drug-resistant strains (*Plasmodium falciparum*) of malaria. The *in vivo* activity and toxicity of the most active compound, 12β-propyldeoxoartemisinin, were comparable to that of arteether.

Introduction

The rapid spread of drug-resistant malaria in Southeast Asia and the threat that resistant strains of *Plasmodium falciparum* will spread to Africa, India, and South and Central America has stimulated a search for new drugs to treat the more than 270 million people infected with malaria.[1] Chinese investigators discovered that artemisinin, **1**, the active principle of *Artemisia annua*, suppresses drug-resistant strains of *P. falciparum* and is effective in treating patients with cerebral malaria (an otherwise fatal condition).[2] A search for new, more effective artemisinin derivatives has therefore been initiated by Chinese investigators and the World Health Organization. Avery et al.[2f] have recently reviewed their structure activity studies of tricyclic analogs of artemisinin. Metabolic studies by Baker et al.[3] demonstrated that β-arteether, **2**, one of the promising new derivatives to emerge from these efforts, was rapidly converted by rat liver microsomes into dihydroartemisinin, **3**. This finding and the fact that the most effective derivatives have been ethers or esters of **3** suggest that they were prodrugs for **3**. The controlled slow formation of **3** is desirable in view of its short half-life in plasma (less than 2 h). In addition, a need exists for artemisinin derivatives that would not serve as prodrugs for **3**.

The successful syntheses of anticancer and antiviral drugs[4] by replacing a carbon–nitrogen bond in nucleosides by a carbon–carbon bond (C-nucleosides) prompted Jung *et al.*[5] and Haynes *et al.*[6] to prepare several 12-alkyldeoxoartemisinins, **4**. Their starting material was artemisinic acid, **5**, a more plentiful constituent of *A. annua*. Typically, these syntheses involved five or six steps. One of the early steps in the conversion of **5** into **4** involved the reaction of an aldehyde, **8**, with a Grignard reagent to produce an epimeric mixture of alcohols. In some syntheses only one epimer was converted into a 12β-alkyldeoxoartemisinin,[5] whereas others yielded mixtures of 12α- and 12β-isomers.[6] In addition to a lack of stereoselectivity, the overall yields were low. For example, Jung et al.[5a] report a 12% yield for introduction of the hydroperoxide group and subsequent acid-catalyzed cyclization of the crude reaction products. The need for multigram quantities of 12β-alkyldeoxoartemisinins for *in vivo* testing prompted us to search for a shorter, stereoselective synthesis of these compounds. We report here the preparation of 12β-allydeoxoartemisinin, **6**, and its conversion into several derivatives. The *in vitro* antimalarial activities of these compounds were determined using two drug-resistant strains (W-2 and D-6) of *P. falciparum*. *In vivo* testing and toxicity studies of the most effective compound, 12β-*n*-propyldeoxoartemisinin, **7**, were carried out, and the results are summarized in this report.



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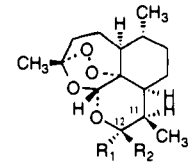

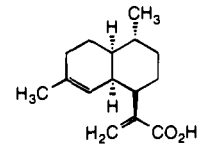

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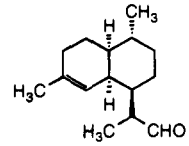

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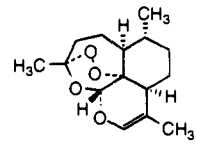

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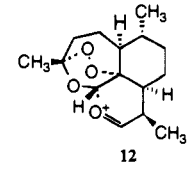
2 R_1 = H: R_2 = OCH_2CH_3
3 R_1 = H: R_2 = OH
4 R_1 or R_2 = CH_2R
6 R_1 = H: R_2 = $^{16}CH_2{}^{17}CH{=}{}^{18}CH_2$
7 R_1 = H: R_2 = $^{16}CH_2{}^{17}CH_2{}^{18}CH_3$
10 R_1 = H: R_2 = CH_2CH_3
11 R_1 = $CH_2CH{=}CH_2$: R_2 = H
13 R_1 = H: R_2 = $CH_2CH_2CH_2OH$
14 R_1 = H: R_2 = OCH_3
15 R_1 = H: R_2 = CH_2CHO
16 R_1 = H: R_2 = CH_2CH_2OH
17 R_1 = H: R_2 = CH_2CH_2OTs
18 R_1, R_2 = H



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Results and Discussion

Kishi *et al.*[7] have employed the reaction of a hemiacetal, allytrimethylsilane and boron trifluoride etherate for the preparation of C-glucosides. When the reaction was performed with **3**, a single isomer of **6** was obtained in addition to 25% of a dehydration product **9**.[8] A comparison of ^1H NMR data for **6** with data reported by Haynes and Vonwiller[6] indicated a β-stereochemistry for the allyl group. However, the criteria Haynes *et al.*

[⊗] Abstract published in *Advance ACS Abstracts*, January 1, 1995.

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employed were not specified, and therefore an independent assignment was sought. Stereochemical assignments at C-12 for esters and ethers of **3** initially relied upon a correlation between the coupling constant $J_{11,12}$ which varies between 8 and 13 Hz for 12α-isomers and between 2 and 6 Hz for 12β-isomers.[9] The assumption that the same relationship would hold for 12α- and 12β-alkyldeoxoartemisinins, however, was shown to be invalid when Haynes and Vonwiller[6] found that $J_{11,12}$ was virtually identical for **11** and **6** (6.1 and 6.3 Hz, respectively). The difference between $J_{11,12}$ for 12α- and 12β-alkyldeoxoartemisinin derivatives and the corresponding 12α- and 12β-ethers of dihydroartemisinin probably can be attributed to differences in the conformation of ring D in the two set of compounds.

A 12β-stereochemistry was assigned to **6** based on the mechanism proposed by Kishi[7] of axial attack by the nucleophilic allyl group on the intermediate oxonium ion **12**, formed from **3** by reaction with a Lewis acid. The assignment agrees with those for **10** and **13** (prepared from **6**) and these compounds synthesized by Haynes[6] and Jung[5] from artemisinic acid. Our stereochemical assignments of **6**, **10**, and **13** interconnect and confirm the assignments previously made by Haynes and Vonwiller.[6]

Comparison of the chemical shifts of H-12 in the ^{1}H NMR spectra of our compounds with those in the literature reveals sufficient differences in the chemical shifts for 12α-alkyl derivatives (δ 3.42−3.49) and the 12β-isomers (δ 4.04−4.30) to allow tentative stereochemical assignments. We employed analogous arguments to distinguish between natural artemisinin and its derivatives with the corresponding 11-epimers.[10]

The facile conversion of **3** to **6** enabled us to prepare sufficient quantities of the latter compound for the transformations described below. Since catalytic hydrogenation of other artemisinin derivatives had been shown to destroy the peroxide group,[11] an alternative procedure for reducing the double bond was needed. Diimide was chosen to effect the reduction, which proceeded in good yield (80%) to give **7**.

The two most promising ethers[8,9] of dihydroartemisinin are **2** and artemether, **14**. Chinese investigators have made extensive use of the latter compound in their studies and have collected considerable clinical data on its efficacy.[2a,e] We therefore prepared the carbon analog, **10**. Ozonolysis of the double bond in **6** yielded the aldehyde **15**, which was not isolated but reduced with sodium borohydride to **16**, which was converted to the tosylate **17**. Reduction of the latter compound with lithium aluminum hydride in cold THF yielded 12β-ethyldeoxoartemisinin, **10**. A comparison of its ^{1}H NMR spectrum with that reported for 12β-ethyldeoxoartemisinin[6] shows they are identical. The *in vitro* antimalarial activities of the above compounds, artemisinin and arteether *versus* the W-2 and D-6 drug-resistant clones of *P. falciparium* are given in Table 1. The IC_{50} values of **2** and **7** were essentially indistinguishable, whereas **10** was approximately half as active as the others. Jung et al.[5b] compared **18** and **1** using clones of W-2 and D-6 and found **18** was 5 times more active than **1**. The IC_{50} of a sample of **18** (preparation given in the Experimental Section) was between that of **10** and **6** (Table 1). We attribute no significance to the observed differences in the activities of **18**, **10**, and **6**.

Table 1. *In Vitro* Antimalarial Acitivity (ng/mL) of Artemisinin and Derivatives against Two Drug-Resistant Clones of *P. falciparum*

	IC_{50}	
compd	W-2	D-6
1	0.32	0.86
2	0.18	0.34
6	0.59	1.07
7	0.16	0.41
10	0.29	1.09
16	0.59	2.04
18[a]	0.48	1.11

[a] Compound **18** was prepared and tested by Jung *et al.*[16] and is given for comparison purposes.

Hydroboration of the double bond in **6** and oxidation produced **13**, previously prepared by Jung *et al.*[5b] using another route.

One motive for synthesizing 12-alkyldeoxoartemisinins was the belief that they would be less reactive than esters and ethers of dihydroartemisinin. The latter compounds are convertible into **3**, which in the presence of weak acids exists in equilibrium with several D-ring-opened species. Thus, the short plasma half-life for dihydroartemisinin[12] might result from reaction of the aldehyde present in such ring-opened species with amino groups present in plasma proteins or the result of intramolecular condensations. Since these reactions could not occur with 12-alkyldeoxoartemisinin derivatives, their *in vivo* half-lives might be significantly longer. The possibility was tested by determining the *in vivo* activity of the most promising compound, **7**, in mice infected with *Plasmodium berghei* and comparing it with that of **2**. The comparison showed that subcutaneous administration of 64 mg/kg/day of **7** in peanut oil resulted in cures for all mice.[13] Compound **2** appeared to be slightly more effective than its carbon analog, **7**.

A second rationale for preparing 12-alkyldeoxoartemisinins was a test of the hypothesis that **3** was responsible for the toxicity observed in dogs[14] given repeated, large doses of **2**. Since these C-glycosides could not be converted into **3** by the enzymes in rat liver microsomes, they might be much safer antimalarial drugs than **2**. The neurotoxicities of **2** and **7** were determined using a mouse model developed by Ager[15] that produces damage similar to that reported by Brewer in dogs.[14] Doses of 64 mg/kg/day were sufficient to cure all mice infected with *P. berghei*; somewhat larger doses produced swelling at the injection site and administration of 400 mg/kg/day resulted in some paralysis and death. Although **7** could not be converted into **3**, Ager *et al.*[15] found that the toxicity of **7** was essentially identical to that of **2**. The doses and protocols employed will be reported by Ager[15] elsewhere.

Summary

A new stereoselective synthesis of 12β-alkyldeoxoartemisinin derivatives from **3** has been developed. The stereochemistry of the critical intermediate **6** was assigned on mechanistic grounds and confirmed by comparing compounds derived from it with those reported in the literature. *In vitro* test results of all our compounds are reported. *In vivo* tests and a toxicity study of **2** and **7** indicate that these properties are essentially identical in both compounds.

Table 2. Summary of ^{13}C NMR Data and Assignments

	compound				
carbon	6	7	10	16	18
1	52.40	52.45	52.51	52.23	52.18
2	24.81	24.90	25.01	24.80	24.74
3	36.69	36.65	36.69	36.49	36.28
4	103.1	103.2	103.1	103.2	104.1
5	89.10	88.95	88.89	89.18	92.12
6	81.07	81.18	81.18	81.01	80.13
7	44.39	44.54	44.56	44.10	44.91
8	25.00	24.75	24.81	24.80	34.06
9	34.58	34.54	34.61	34.39	37.32
10	37.56	37.53	37.54	37.48	37.32
11	30.30	30.33	30.38	30.30	27.95
12	74.67	75.50	77.57	75.15	66.21
13	13.08	13.11	13.15	12.89	13.08
14	20.29	20.26	20.33	20.15	20.28
15	26.19	26.20	26.27	26.02	26.08
16	34.33	31.50	22.42	31.57	
17	136.4	20.80	12.20	62.76	
18	116.0	14.04			

Experimental Section

All NMR spectra were measured with a Varian Gemini-300 NMR spectrometer. Rotations were measured with a Perkin-Elmer 241MC polarimeter. CI-MS were determined with a Finnigan 4600 mass spectrometer. Elemental analyses were performed by Galbraith Laboratory, Inc., Knoxville, TN. All solvents were purified prior to use. Allyltrimethylsilane and the other reagents were purchased from the Aldrich Chemical Co.

12β-Allyldeoxoartemisinin (6). A scaled up version of the procedure given in ref 8 was employed to prepare several batches of **6** in the following manner. Allyltrimethylsilane (5 mL, 0.048 mol) and boron trifluoride etherate (6.0 mL, 0.048 mol) were added to a cold (−55 °C) solution of **3** (8.0 g, 0.028 mol) in CH_2Cl_2 (180 mL) under argon. The solution was allowed to warm to −5 °C over 6 h. Water (100 mL) and $CHCl_3$ (200 mL) were added, and the organic layer was separated. It was washed with water, dried over Na_2SO_4, and concentrated. The mixture was purified by flash chromatography on silical gel to afford 4.3 g of **6** as a white solid, mp 76−78 °C. In addition 1.7 g (23%) of **9** was obtained. The physical properties of **6** and **9** were identical to those reported in ref 8.

2β-*n*-Propyldeoxoartemisinin (7). A solution of **6** (3.0 g, 0.0097 mol), hydrazine monohydrate (120 mL), and ethanol (700 mL) was heated at 55°C, with stirring, for 6 h. The ethanol was removed under reduced pressure and $CHCl_3$ (300 mL) added. The organic layer was separated, washed with water, and dried (Na_2SO_4). The crude product was purified by flash chromatography on silica gel to yield **7** (2.5 g, 80%) as a white solid: mp 56−57 °C; $[\alpha]_D$ +83° (*c* 0.47, $CHCl_3$), +78° (*c* 0.47, EtOH); ^1H NMR ($CDCl_3$) δ 5.31 (1H, s, H-5), 4.26 (1H, ddd, H-12, *J* = 4.0, 6.1, 9.6 Hz), 1.42 (3H, s, H-15), 0.96 (3H, d, H-14, *J* = 5.9 Hz), 0.94 (3H, t, H-18, *J* = 7.0 Hz), 0.85 (3H, d, H-13, *J* = 7, 6 Hz); ^{13}C NMR assignments are listed in Table 2; CI-MS (NH_3) 328 $(M + NH_4)^+$, 100; 311, 68; 265, 43. Anal. Calcd for ($C_{18}H_{30}O_4$) C, 69.64; H, 9.74. Found: C, 69.79; H, 9.73.

12β-(2-hydroxyethyl)deoxoartemisinin (16). A solution of **6** (300 mg, 0.97 mmol) in dry CH_2Cl_2 (100 mL) was flushed with N_2 and then subjected to a stream of O_3 at −78 °C for 30 min. Residual O_3 was then flushed from the solution with N_2, and the solvent was removed under reduced pressure. Methanol−THF (100 mL of a 9:1 mixture) was added, and the solution was treated with excess sodium borohydride (2.0 g) at 0 °C for several hours. The solution was concentrated under reduced pressure, water and $CHCl_3$ were added, and the organic layer was separated, dried, and concentrated. The product was purified by flash chromatography on silica gel with hexane−acetone (7:3) to yield **16** (240 mg, 79%): mp 103−105 °C; $[\alpha]_D$ +66° (*c* 0.45, $CHCl_3$), +80° (*c* 0.45, EtOH): ^1H NMR ($CDCl_3$) δ 5.37 (1H, s, H-5), 4.45 (1H, ddd, H-12; *J* = 4.1, 6.1, 9.6 Hz), 3.83 (2H, m, H-17), 1.41 (3H, s, H-15), 0.96 (3H, d, H-14; *J* = 5.4 Hz), 0.87 (3H, d, H-13; *J* = 7.6 Hz); data on ^{13}C spectra are given in Table 2; CI-MS (NH_3) 330 $(M + NH_4)^+$ (23), 313 (43), 295 (100). Anal. Calcd for ($C_{17}H_{28}O_5$) C, 65.36; H, 9.03. Found: C, 65.09; H, 9.08.

12β-ethyldeoxoartemisinin (10). The tosylate **17** was prepared by reacting **16** (62 mg, 0.20 mmol), *p*-toluenesulfonyl chloride (190 mg, 1.0 mmol), pyridine (1 mL), and (dimethylamino)pyridine (60 mg) in methylene chloride (8 mL) overnight at room temperature. The crude reaction product was purified by chromatography on silica gel with hexane:ethyl acetate (9:1) to yield **17**, an oil (80 mg, 85%), which was reduced with 1 equiv of $LiAlH_4$ in cold THF. After chromatography on silica gel, **10** was isolated as a solid (30 mg, 65%): mp 60−62 °C; $[\alpha]_D$ +75° (*c* 0.30, $CHCl_3$), +70° (*c* 0.30, EtOH); ^1H NMR ($CDCl_3$) δ 5.28 (1H, s, H-5), 4.05 (1H, ddd, H-12; *J* = 4.1, 6.0, 9.5 Hz), 1.43 (3H, s, H-15), 1.03 (3H, t, H-17; *J* = 7.3 Hz), 0.96 (3H, d, H-14, *J* = 5.9 Hz), 0.86 (3H, d, H-13; *J* = 7.6 Hz); ^{13}C spectra and assignments are given in Table 2; CI-MS (NH_3) 314 $(M + NH_4)^+$ (29), 297 (17), 279 (17), 251 (43). The data agree with those reported by Haynes and Vonwiller.[6]

Acknowledgment. We wish to thank Msrs. Noel Whittaker and Wesley White of NIDDK for CI-MS and Dr. Steven Andersen at the Walter Reed Army Institute of Research for *in vitro* testing. We also wish to thank Dr. Arba L. Ager, Jr., of the Center for Tropical Parasitic Diseases, University of Miami, for *in vivo* testing which was supported by the Walter Reed Army Institute of Research. Finally, we also acknowledge the generosity of WHO for providing us with artemisinin and β-arteether.

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JM9405856