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SYNTHESIS OF 11-[^{3}H]-ARTEETHER, AN EXPERIMENTAL ANTIMALARIAL DRUG

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SUMMARY

The triphenylphosphine hydrobromide-catalyzed addition of a proton from ethanol to anhydrodihydroartemisinin (**5**) has been employed for the synthesis of **2** and **4**. Use of 1.5 equivalents of EtO^{2}H or EtO^{3}H yielded 11-[^{2}H]- or 11-[^{3}H]-β-arteether as the major product.

Keywords: Arteether, Artemisinin, Anhydrodihydroartemisinin, Malaria

INTRODUCTION

Artemisinin (**1**) (the active principle of the medicinal herb, *Artemisia annua*) was isolated by Chinese investigators,[1] who demonstrated that it was effective in treating patients with cerebral malaria, otherwise fatal, as well as those infected with drug-resistant strains of *Plasmodium falciparum*. Their success prompted them and others to prepare a variety of derivatives.[1] One of the most promising derivatives was β-arteether (**2**) [2] which was several times more active than **1**. However, large doses of **2** were found to cause neurological side effects in dogs and mice.[3] Radiolabelled **2** is required to aid in identifying the toxic agent responsible for these side effects. Several preparations of labelled **2** have been described. A ^{14}C labelled sample was prepared from dihydroartemisinin (**3**) and ^{14}C-ethanol,[4a] and ^{3}H labelled material was prepared by reduction of the lactone moiety in artemisinin with NaB^{3}H$_4$, followed by etherification.[4b,c] Since Baker *et al.* demonstrated that O-dealkylation was a major metabolic route of **2**,[5] it was anticipated that a

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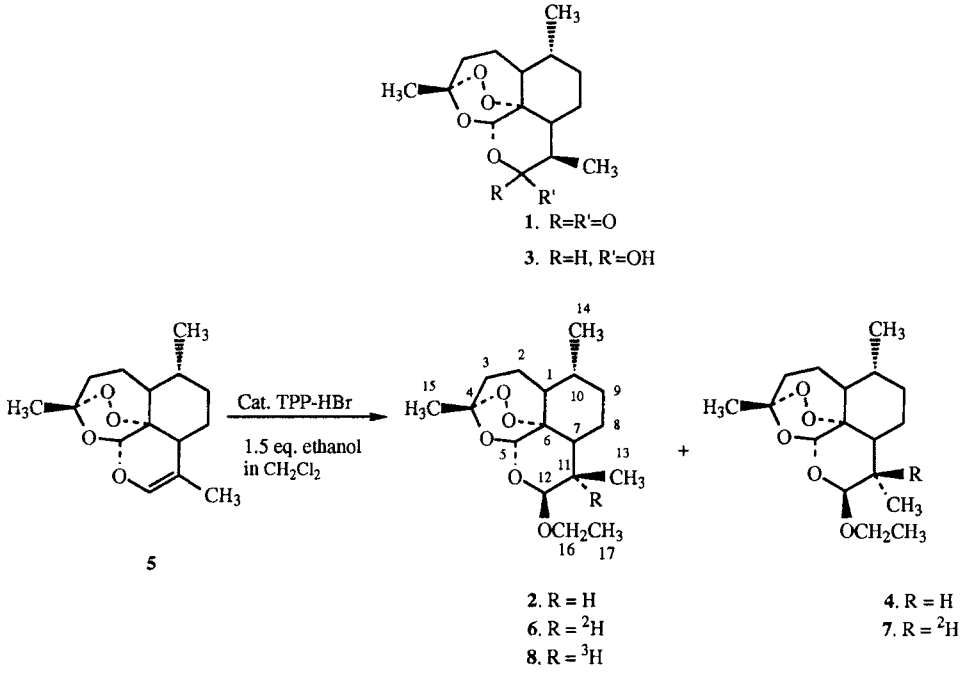
labelled ethyl group would rapidly be lost. It was also feared that a ^3H atom on C-12, a labile position, would be lost during *in vivo* metabolism, isolation and purification of metabolites. We previously reported a procedure for preparing 14-[^2H]-arteether,[6] however the starting material, a microbial transformation product of arteether, is not readily accessible. This new procedure uses a readily available intermediate to incorporate a tritium label on C-11, where it should be stable to enzymatic degradation and /or exchange under physiological conditions (Scheme1).



Scheme 1: Synthesis of Arteethers from Anhydrodihydroartemisinin (**5**).

Franck *et al.* [7] reported a deuteron could be introduced β to the oxygen of an enol ether by employing catalytic quantities of triphenylphosphine hydrobromide (TPP-HBr) and EtO^2H. We investigated TPP-HBr catalyzed additions of alcohols to anhydrodihydroartemisinin (**5**),[8] and found that a 1:3 mixture of **4** and **2** was obtained with one to two equivalents of ethanol. This method was utilized to prepare 11-[^2H] and [^3H]-β-arteethers.

RESULTS AND DISCUSSION

Anhydrodihydroartemisinin (**5**) was prepared from **3** by treatment with phosphorous pentoxide.[9] Reaction of **5** with 1.5 equivalents of EtO^2H in methylene chloride and catalytic quantities of TPP-HBr converted **5** into a 1:3 mixture of **7** and **6**. The mixture was separated on a reverse phase

HPLC by eluting with methanol:water (85:15). The deuterium content of **6** was measured by integrating the H-11 signal at δ = 2.6 ppm and the H-12 signal at δ = 4.79 ppm. A chromatographic procedure was sought as an alternative to HPLC in purifying **8**. The reaction mixture of **8** was purified first by TLC with hexane: ethyl acetate (9:1). The resulting product was subjected to a second TLC purification with chloroform: isopropyl ether (97:3). Addition of a deuteron to an enol ether (glucal) occurs *via* an Ad_E2 mechanism, *i.e.* a kinetically controlled deuteronation; the stereochemistry of the alcohol addition is controlled by a kinetic anomeric effect. Bolitt and Mioskowski [10] have shown that the behavior of TPP-HBr differs from that of Brönsted or Lewis acids. For example, they described the preparation of 2-desoxy-D-glycopyranosides from glucals without Ferrier rearrangement.

EXPERIMENTAL

Materials and Instrumentation. Diethoxydimethylsilane, phosphorous pentoxide, deuterated ethanol (>99.5% D), triphenylphosphine hydrobromide, and absolute ethanol were purchased from Aldrich and used without purification. Tritiated water (specific activity = 1.0 mCi/mL) was from NEN. All other reagents were ACS grade or the highest quality material available. Dried dichloromethane was prepared by refluxing in P_2O_5 for a few hours and distilled off before use. ^{1}H-NMR was run at 300 MHz and ^{13}C-NMR at 75 MHz on a Varian Gemini 300. FT-IR was measured on Bio-Rad FTS-45, and CI-MS on a Finnigen 4600 Mass Spectrometer. Elemental analysis was performed by Galbraith Laboratory, Inc., Tennessee. Radioactivity was measured by a Beckman LS3800 liquid scientillator.

Radioactive Ethanol ($CH_3CH_2O^3H$).[11] To a mixture of diethoxydimethylsilane (7.4g, 0.05 mole) and 1.0 g of radioactive water (NEN, 1.0 mCi/mL, e.g. 0.018 mCi/ mmole) was added 50 uL of concentrated aqueous HCl . The reaction mixture was stirred at rt. for 15 min. and the ethanol distilled from the flask (4.6 g, 0.010 mCi/ mmole).

Anhydrodihydroartemisinin (5). To a solution of dihydroartemsinin (1.5 g, 5.28 mmole) in 200 mL of dried CH_2Cl_2 was added 1.5 g of P_2O_5 powder at rt. The reaction mixture was stirred for 30 min., and poured into water. The organic layer was separated, washed with 1% aqueous $NaHCO_3$, dried, filtered, and concentrated. The crude product was purified by flash chromatography (Hexane:ether=15:1) to afford 1.1 g of **5** (80%). mp 94-96 °C (Lit.[9] mp 95-97°C); $[\alpha]_D$= +130° (*c* 0.85, $CHCl_3$), +120° (*c* 0.85, EtOH); ^{1}H-NMR ($CDCl_3$) δ 6.17 (1H, s,

C_{12}-CH=) , 5.51 (1H, s, C_5-CH), 2.38 (1H, ddd, J=13.9, 13.9, 3.9 Hz, C_3-CH_αH), 2.3-1.0 (10H, overlapping, carbon skeleton protons), 1.57 (3H, s, C_{13}-CH_3), 1.41(3H, s, C_{15}-CH_3), 0.96 (3H, d, J=5.1 Hz, C_{14}-CH_3).

β-Arteether (2) and 11-epi-β-Arteether (4).

In a 100 mL round bottom flask were placed anhydrodihydroartemisinin (318 mg, 1.2 mmole), triphenylphosphine hydrobromide (21.0 mg, 0.06 mmole), absolute ethanol (0.11 mL, 1.8 mmole) and 36 mL of dried CH_2Cl_2. The reaction mixture was stirred at rt. for 3 h and poured into water. The organic phase was washed with 1% aqueous $NaHCO_3$, dried, filtered and concentrated. The crude product was purified by flash chromatography (hexane:ethyl acetate = 94:6) to afford a mixture of β-arteether (**2**) and 11-epi-β-arteether (**4**) (165 mg, 3:1). The mixture was separated by reverse phase HPLC (methanol:water =85:15) to provide 110 mg of **2** (29%) and 34 mg of **4** (9%).

2: mp 81-83 °C (Lit.[2] 80-82 °C); $[\alpha]_D$= +150° (*c* 0.50, $CHCl_3$), +140° (*c* 0.50, EtOH). ^{1}H- and ^{13}C-NMR spectral data were in accord with those reported.[2]

4: mp 60-62 °C; $[\alpha]_D$= +140° (*c* 0.50, $CHCl_3$), +130° (*c* 0.50, EtOH), ^{1}H-NMR ($CDCl_3$) δ 5.46 (1H,s, C_5-CH), 4.98 (1H,d, J=5.3 Hz, C_{12}-CH), 3.85, 3.51 (2H, m C_{16}-CH_2), 2.24 (1H, ddd, J=13.9, 13.9, 3.9 Hz, C_3-CH_αH), 2.02 (1H, ddd, J=13.9, 3.9, 3.9 Hz, C_3-CHH_β), 2.0-1.0 (10H, overlapping, carbon skeleton protons), 1.41 (3H,s, C_{15}-CH_3), 1.24 (3H, t, J=7.0 Hz, C_{17}-CH_3), 1.18 (3H,d, J=7.0 Hz, C_{13}-CH_3), 0.95 (3H, d, J=6.1Hz, C_{14}-CH_3); ^{13}C-NMR (Table1); FT-IR (KBr) 2960, 1050, 980, 925 cm^{-1} ; CI-MS (NH_3): 330 [$(M+NH_4)^+$, 5 %], 284 [$(M+NH_4)^+$ -CH_3CH_2OH, 28%], 267 [$(M+NH_4)^+$ -CH_3CH_2OH - NH_3, 100%]; Anal. calcd for $C_{17}H_{28}O_5$; C, 65.36; H, 9.03. Found C, 65.33; H, 9.05.

11-[^{2}H]-β-Arteether (6) and 11-[^{2}H]-11-epi-β-Arteether (7).

These compounds were prepared using deuterated ethanol and purified as described above.

6 (30%): mp 81-83 °C; $[\alpha]_D$= +150° (*c* 0.50, $CHCl_3$), +140° (*c* 0.50, EtOH), ^{1}H-NMR ($CDCl_3$) δ 5.41 (1H, s, C_5-CH), 4.79 (1H, s, C_{12}-CH), 3.83, 3.43 (2H, m, C_{16}-CH_2), 2.37 (1H, ddd, J=14.1, 14.1, 3.9 Hz, C_3-CH_αH), 2.05 (1H, ddd, J=14.1, 3.9, 3.9 Hz, C_3-CHH_β),

2.0-1.0 (9H, overlapping, carbon skeleton protons), 1.44 (3H, s, C_{15}), 1.18 (3H, t, J=7.0 Hz, C_{17}-CH_3), 0.95 (3H, d, J=6.1Hz, C_{14}-CH_3), 0.90 (3H,s, C_{13}-CH_3); ^{13}C-NMR (Table 1); CI-MS (NH_3): 331 [$(M+NH_4)^+$, 10%], 284 [$(M+NH_4)^+$-CH_3CH_2OD, 28%], 267 [($M+NH_4)^+$ -CH_3CH_2OD- NH_3, 100%]; Anal. calcd for $C_{17}H_{27}DO_5$; C, 65.15; H, 8.68. Found C, 65.27; H, 8.88.

7 (10%): mp=60-62°C, $[\alpha]_D$=+140° (*c* 0.50, $CHCl_3$), +130° (*c* 0.50, EtOH); ^{1}H-NMR ($CDCl_3$) δ 5.46 (1H, s, C_5-CH), 4.98 (1H, s, C_{12}-CH), 3.92, 3.58 (2H, m, C_{16}-CH_2), 2.30 (1H, ddd, J=14.0, 14.0, 3.9 Hz, C_3-C*H*αH), 2.03(1H, ddd, J=14.0, 3.9, 3.9 Hz, C_3-CH*H*β), 2.0-1.0 (9H, overlapping, carbon skeleton protons), 1.42 (3H, s, C_{15}-CH_3), 1.24 (3H, t, J=7.1 Hz, C_{17}-CH_3), 1.17 (3H, s, C_{13}-CH_3), 0.95 (3H, d, J=5.8 Hz, C_{14}-CH_3); ^{13}C-NMR (Table 1); FT-IR (KBr) 2960, 1050, 988, 924 cm^{-1}; CI-MS (NH_3): 331[$(M+NH_4)^+$, 10%], 284 [$(M+NH_4)^+$-CH_3CH_2OD, 40%], 267 [$(M+NH_4)^+$ -CH_3CH_2OD-NH_3, 100%]; Anal. calcd for $C_{17}H_{27}DO_5$; C, 65.15; H, 8.68. Found C, 65.30; H, 8.90.

Radioactive 11-[^{3}H]-β-Arteether (8). The compound was prepared as described above starting from the radioactive ethanol (0.010 mCi/mmole). The purification was modified as

Table 1 : ^{13}C-MNR Assignment of Arteethers

Carbon #	2	6	4	7
1	52.81	52.63	51.88	51.85
2	24.80	24.78	24.80	24.78
3	36.60	36.53	36.60	36.59
4	104.0	104.0	102.9	102.8
5	87.90	87.86	89.16	89.14
6	81.15	81.16	81.70	81.14
7	44.65	44.48	46.69	46.59
8	*24.63*	*24.51*	*31.69*	*31.59*
9	34.78	34.73	34.49	34.47
10	37.56	37.50	37.35	37.34
11	*31.01*	*30.9(wk)*	*40.02*	*40.0(wk)*
12	*101.7*	*101.6*	*102.3*	*102.3*
13	*13.14*	*13.01*	*19.55*	*19.42*
14	20.36	20.46	20.14	20.12
15	26.31	26.30	26.04	26.03
16	63.78	63.76	64.18	64.16
17	15.31	15.31	15.31	15.29

followed: The crude product was first purified by preparative TLC (Analtech, 20x20 cm, 500 μm thickness) with hexane : ethyl acetate (9:1) and rechromatographed on TLC with $CHCl_3$: isopropyl ether (97:3) to give **8** (0.0038 mCi/mmole).

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