SYNTHESIS OF 14-[^{2}H]ARTEETHER, AN EXPERIMENTAL ANTMALARIAL DRUG

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SUMMARY

A reaction sequence employing a fungus mediated oxidation of arteether, an experimental antimalarial drug, was used to introduce a hydroxyl group on C-14. The hydroxyl group was replaced by a deuterium by preparing the tosylate and reductively cleaving it with sodium borodeuteride in DMSO. A reaction sequence to introduce a second label was also demonstrated it involved oxidation of the alcohol, with catalytic quantities of tetra-n-propylammonium perruthenate, followed by reduction with sodium borodeuteride to regenerate the alcohol. Use of both reactions sequences yield arteether containing two deuterium.

Key words: Arteether, Artemisinin, Qinghaosu, malaria

INTRODUCTION

In a continuing search for chemotherapeutic agents for treating patients with drug resistant strains of malaria, recent efforts focused on derivatives of artemisinin, **1**, the active principle of the Chinese medicinal drug "Qinghao" (1). Structure-activity studies demonstrated that the endo peroxide was essential for biological activity (2). Water and oil soluble derivatives of dihydroartemisinin were prepared and tested (3) in an effort to obtain the most active antimalarial drug. One of the most promising compounds to emerge from these studies was arteether, **2**, the ethyl ether of dihydroartemisinin. Its metabolism by rat-liver microsomes was examined by Baker et al. (4), who demonstrated the major metabolic pathway involved O-dealkylation to yield dihydroartemisinin, **3**. Other mammalian metabolites were also identified.

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Hufford et al. identified several products from the microbial metabolism of **2** and found that the peroxide bridge, required for biological activity, had been destroyed (5).

In our efforts to introduce additional functional groups into artemisinin derivatives, we employed the fungus *Beauveria sulfurescens* to oxidize C-14 of **4**, a derivative of dihydroartemisinin, to yield **5**. The peroxide group, necessary for antimalarial activity, was retained in the hydroxylated derivative (6). Since arteether is still an experimental drug, material containing a metabolically stable radiochemical label was required for a variety of studies. We have therefore developed reaction sequences described below to make use of **6** to introduce one or two deuterium atoms into the 14-methyl group by a route that can also be employed for tritium.

Artemisinin and its derivatives have been labelled by several groups of investigators on positions that were later found to be metabolically unstable. For example, Gu et al (7) introduced a tritium atom on C-12 of dihydroartemisinin by reducing the carbonyl of artemisinin with sodium borotritide. Since the hemiacetal is in equilibrium with the aldehyde, oxidation of the latter would result in loss of the tritium atom. A ^{14}C labelled methyl ether (artemether) was prepared from dihydroartemisinin and $^{14}CH_3OH$ (8). However, since Baker et al. demonstrated that rat-liver microsomes rapidly O-dealkylated **2**, this label would be rapidly lost *in vivo* and it can not be used to identify late or secondary metabolites. Their presence was noted by Baker et al. and currently there is interest in identifying these materials.

RESULTS AND DISCUSSION

Success in introducing a C-14 hydroxy group on the N-phenylcarbamoyl dihydroartemisinin in low yield, prompted us to examine the ability of *B. sulfurescens* to hydroxylate **2**. That study demonstrated that **6** was formed from **2** in approximately 20% yield and could therefore be employed as an intermediate for a variety of studies (9). Its structure was assigned from mass spectrometric and n.m.r. (2D ^{1}H and ^{13}C) measurements. We have therefore developed one reaction sequence to replace the hydroxy group in **6** with a deuterium (or

tritium) atom. A reaction sequence to introduce a second deuterium (or tritium) atom was also demonstrated.

The tosylate, **7**, was prepared from **6** using tosyl chloride and catalytic quantities of 4-dimethylaminopyridine in methylene chloride. While tosylates are usually reductively cleaved with $LiAlH_4$, use of a milder reducing agent was necessary to avoid destruction of the peroxide. Furthermore, $NaB(^3H)_4$ is commercially available while $LiAl(^3H)_4$ is not. Hutchins et al. (10) demonstrated that nucleophilic substitution reactions were markedly accelerated in polar aprotic solvents such as DMSO. These conditions did not greatly enhance reactions that proceed by other mechanisms. Since the peroxide bridge was unaffected during the reduction of **1** to **2**, sodium borodeuteride in DMSO was employed to displace the tosylate moiety to yield 14-^{2}Harteether, **8**.

If arteether of high specific activity would be required, it could be prepared by including the short oxidation-reduction sequence described below. Oxidation of **6**, using catalytic amounts of tetra-n-propylammonium perruthenate and N-methylmorpholine N-oxide in methylene chloride (11) yields **9**. Reduction of the aldehyde with sodium borodeuteride (tritide) yields **10** containing a deuterium or tritium. When **10** is converted into its tosylate and reduced as described above, the product should contain <u>two</u> metabolically stable deuterium (tritium) atoms.

CONCLUSION

A microbially mediated oxidation was used to introduce a hydroxyl group on an unactivated methyl group in **2**. This metabolite was employed to prepare arteether containing one deuterium (tritium) atom. A second deuterium (tritium) can be introduced by an initial oxidation-reduction of the 14-hydroxyl as described followed by preparation and reduction of the tosylate.

EXPERIMENTAL

Microbial conversion of arteether:

A 1 L Erlenmeyer flask containing 250 mL of medium (Sabouraud Liquid Broth Modified Antibiotic Medium 13, BBL, Cockeysville MD) was innoculated with

Beauvaria sulfurescens (ATCC-7159) grown on a slant made from the same medium containing 2% agar. The flask was shaken (200 RPM) at 27°C for 72 hrs. Aliquots of this culture were employed to innoculate twelve 1 L Erlenmeyer flasks containing 250 mL of medium and approximately 100 μg of arteether. The flasks were shaken (200 RPM) at 27°C for 48 hrs., the mycelia collected by filtration, and resuspended in buffer (200 mL of 0.1 M KH_2PO_4, pH 7.5). Arteether (40 mg in 0.4 mL of ethanol) was added to each flask. The flasks were shaken for 3 days, the mycelia removed by filtration and the combined filtrates were extracted with ethyl acetate (3 x 600 mL). The extracts were dried (Na_2SO_4) and concentrated.

Isolation and characterization of 14-hydroxyarteether (6):

The above residue was purified by flash chromatography on silica gel by successively eluting the column (2.5 x 19 cm) with 500 mL of 15 % ethyl acetate and 85% hexane; 250 mL of 20% ethyl acetate and 80% hexane and 500 mL of 30% ethyl acetate and 70% hexane. The eluant was collected in 8 mL fractions. In addition to recovered starting material (100 mg) four metabolites were isolated. The last metabolite eluted from the column was 14-hydroxyarteether (fraction 86-123, 110 mg, 29% yield), $[\alpha]_D^{25°C}$ +109.6° (c 0.12, $CHCl_3$); CIMS m/z 346 ($M^+ + NH_4^+$); ^1H NMR (500 MHz, $CDCl_3$) δ 0.91 (3H, d, Me-13); 1.10 (1H, m, H-9a); 1.18 (3H, dd, O-CH_2-$\underline{CH_3}$); 1.44 (3H, s, Me-15); 1.44 (1H, OH); 1.47 (1H, m, H-10); 1.55 (1H, m, H-2a); 1.60 (1H, m, H-1a); 1.80 (1H, m, H-9e); 1.82-1.87 (2H, m, H-8); 1.87 (1H, m, H-2e); 2.05 (1H, m, H-3e); 2.39 (1H, m, H-3a); 2.62 (1H, m, H-11); 3.47 (2H, m, O-$\underline{CH_2}$-); 3.58 (1H, dd, H-14); 3.73 (1H, q, H-14); 3.86 (1H, m, O-$\underline{CH_2}$-CH_3); 4.80 (1H, d, H-12); 5.42 (1H, s, H-5).

Preparation of Tosylate ester (7):

To a solution of **6** (15 mg, 0.018 mM) in dry methylene chloride (1 mL) was added tosyl chloride (10.5 mg, 0.055 mM) and 4-dimethylaminopyridine (DMAP)(6 mg, 0.049 mM). The solution was refluxed for 2 hrs. and water (1 mL) was added. The methylene chloride was evaporated with a stream of N_2 and the residue was extracted into ethyl acetate; the combined extracts were washed with Na_2HCO_3, water, 1N HCl and water; dried over anhydrous Na_2SO_4 and concentrated. The tosylate was purified by chromato-graphy on a 250μ silica gel glass plate using 20% ethyl acetate 80% hexane. The uv-absorbing band (R_f 0.28) was cut out and

the tosylate eluted with ethyl acetate. The washings were concentrated to yield **7** (approximately 5 mg). ^1H NMR (300 MHz, $CDCl_3$): δ 1.16 (3H, t, O-CH_2-$\underline{CH_3}$); 1.42 (3H, s, CH_3-15); 2.46 (3H, s, Ar-CH_3); 3.97-4.07 (2H, m, H-14); 4.77 (1H, d, H-12); 5.33 (1H, s, H-5); 7.26-7.80 (4H, m, Ar-H).

Synthesis of 14-^2H arteether (8).

Approximately 5 mg (0.010 mM) of **7** in DMSO was treated with excess $NaBD_4$ at 80-85°C for 3 hrs. The reaction was followed by TLC. When the starting material was no longer detected by TLC, water was added and the solution was extracted with ethyl acetate. The combined extracts were washed with water, dried (Na_2SO_4) and concentrated. The crude reaction mixture was purified on a 250μ silica gel thin layer plate with 10% ethyl acetate, 90% hexane. A band (R_f 0.38) was cut out and the compound eluted with ethyl acetate and concentrated to yield **8** (approximately 2 mg). CIMS: m/z 331 ($M^+ + NH_4^+$). Its ^1H NMR ($CDCl_3$) spectrum was identical to that obtained from a sample of **2**, with the exception of the integral for 14-CH_3.

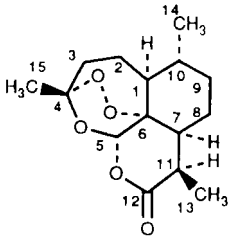
Oxidation of 14-hydroxyarteether (6):

To a solution of **6** (6 mg, 0.018 mM) in dry methylene chloride (0.5 mL) was added 4-methylmorpholine N-oxide (MNO) (3.3 mg, 0.028 mM) and molecular sieves (4 mg of 4 A powdered material). The reaction mixture was stirred for 30 min. and a trace quantity of tetra-n-propylammonium perruthenate (TPAP) was added. The reaction mixture was stirred at room temperature and the starting material had been consumed (monitored by TLC) after 2 hrs. The reaction mixture was filtered and the filtrate was concentrated. The residue was chromatographed on a silica gel thin layer plate using 20% ethyl acetate and 80% hexane. A band (R_f 0.34) was cut out and eluted with ethyl acetate to yield **9** (3 mg). CIMS: m/z 344 ($M^+ + NH_4^+$); ^1H NMR (in $CDCl_3$) δ 0.92 (3H, d, CH_3-13); 1.19 (3H, O-CH_2-$\underline{CH_3}$); 1.45 (3H, s, CH_3-15); 4.81 (1H, d, H-12); 5.38 (1H, s, H-5) 9.53 (1H, s, C$\underline{H}$O).

Reduction of 9 with $NaBD_4$:

To a solution of **9** in methanol (2 mg, 0.0061 mM, in 0.5 mL) containing $CeCl_3$ at -78°C was added sodium borodeuteride (excess). The solution was stirred

for 4 hrs and allowed to warm to room temperature. A drop of acetic acid and 0.5 mL of water were added; the mixture was extracted with ethyl acetate. The combined extracts were washed with water, dried (Na_2SO_4) and concentrated to afford **10**, approximately 2 mg. CIMS m/z 347 ($M^+ + NH_4^+$). ^1H NMR (in $CDCl_3$): The spectrum was identical to that of **6** with the exception of the integral for H-14.



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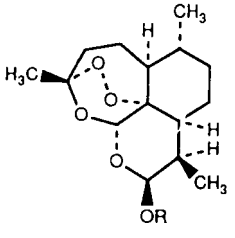
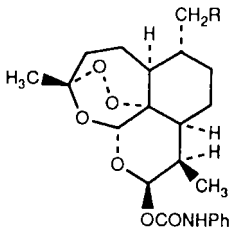
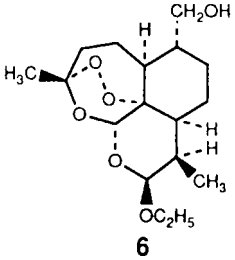


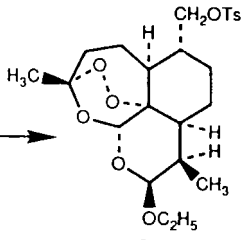
2 R=C_2H_5
3 R=H



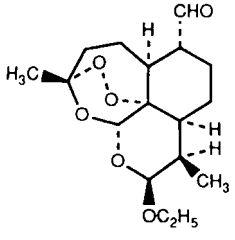
4 R=H
5 R=OH



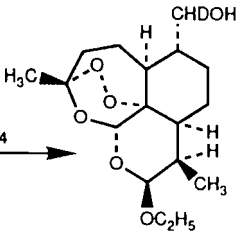
6



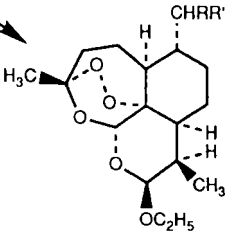
7



9



10



8 R=D, R'=H
11 R=R'=D

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