Deuterated Antimalarials: Synthesis of Trideutero- Artemisinin, Dihydroartemisinin, and Arteether

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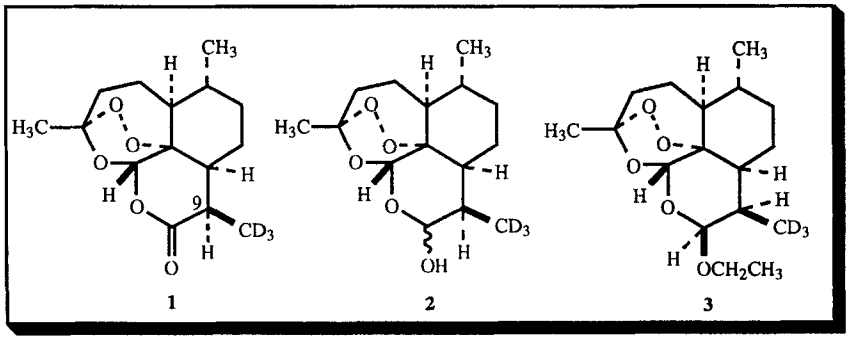
Summary

The stereoselective total synthesis of the antimalarial agents artemisinin-d_3 (**1**), dihydroartemisinin-d_3 (**2**), and arteether-d_3 (**3**), incorporating ^2H at C-16 (C-9 methyl) is reported.

Key Words: malaria, artemisinin, deuterium, dihydroartemisinin, arteether

Introduction

In recent years, the number of cases of malaria untreatable by conventional medicines has risen dramatically, in large part due to the spread of drug resistant strains of *Plasmodium Falciparum*. (1-4). For this reason, the development of antimalarials well-suited to effectively combat *P. Falciparum* , one of the major drug-resistant parasites responsible for malaria, has become of paramount importance. The discovery of artemisinin, of Chinese herbal origin, with properties superior to the more traditional antimalarials, was instrumental in defining a new approach to the chemotherapy of drug-resistant malaria (5-7).



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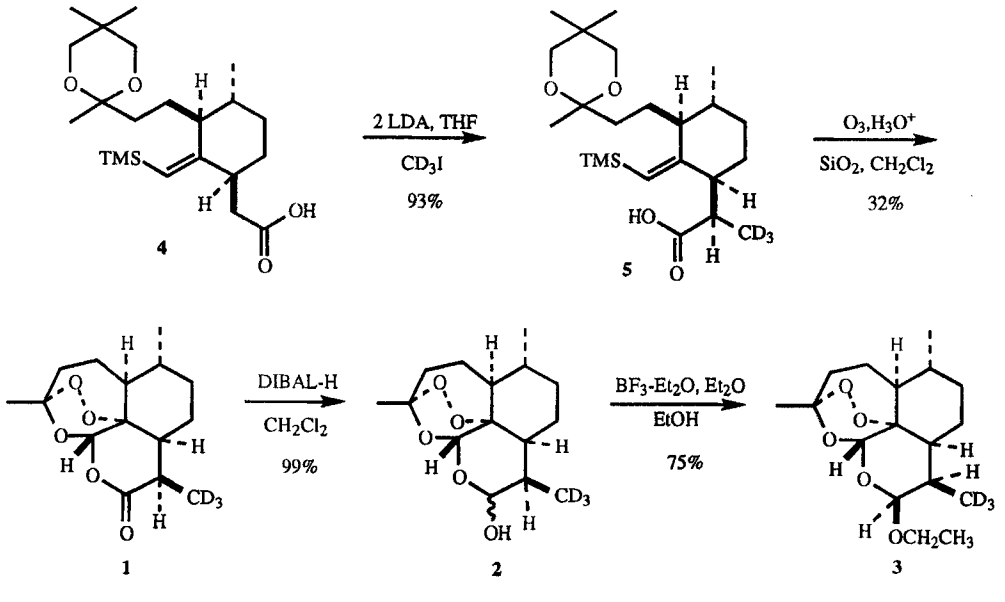
The subsequent evolution of artemisinin-type compounds into a therapeutically useful regimen has offered solutions to such problems as poor oral availability, water solubility, and short plasma half-life, resulting in the compounds dihydroartemisinin, **2**, and arteether, **3**, as well as others (8-11). Studies devoted to drug stability and metabolism should profit considerably from isotopic substitution; furthermore, synthesis of the desired compounds was straightforward, providing the title compounds in good yield.

Results and Discussion

The ease of construction of these compounds was enhanced by the fact that we had access to a large amount of a total synthesis intermediate, ketal acid **4** (12) (Scheme I). Alkylation of **4** with CD_3I gave acid **5** as a single stereoisomer in 93% yield. No traces of epimeric (9α-CH_3) material were observed by thin layer chromatography or NMR in the unlabelled series, and we expected the labelled series to behave similarly; in fact, no epimeric material was found, and ozonolysis of the crude labelled mixture failed to produce traces of labelled 9-α-CD_3 artemisinin as well. (13) Ozonolysis followed by acid catalyzed cyclization afforded artemisinin-d_3 **1** in 32% yield. Reduction of **1** with diisobutylaluminum hydride provided the desired lactol **2** (as a mixture of α and β epimers) in excellent yield. Finally, formation of arteether-d_3 (**3**) with ethanol and boron trifluoride etherate proceeded nicely (75%). Further evidence for the β-configuration at C-9 was

SCHEME I



found in the ^1H NMR spectrum of **3**: the H9-H10 coupling constants for unlabelled β-arteether and α-arteether are 9.2 and 3.6 Hz, respectively. (9) The H9-H10 coupling in **3** was 3.6 Hz, verifying the β-configuration at both C-9 and C-10.

In short, we report the stereoselective synthesis of three potent antimalarials bearing ^2H at C-16 (C-9 methyl). The potential of these compounds resides in their inherent ability to provide elusive information in metabolic studies, ultimately allowing faster progress toward a more refined and potent antimalarial drug. Exploitation of the isotopic labelling via LC-MS determination of metabolites obtained from rat liver perfusate is in progress, and will be reported in due course.

Experimental

All solvents were purchased as HPLC grade, and where appropriate were distilled from CaH_2 prior to use. Solvent and reagent transfers were accomplished via dried syringe, and all reactions were routinely conducted under an inert atmosphere, unless otherwise indicated. Flash chromatography was accomplished using silica gel (Whatman 60, 230-400 mesh). Preparative thin-layer chromatography utilized 1-,1.5-, or 2-mm-thick Analtech Uniplates with F-256, and 250-μm silica gel thin-layer chromatography plates were also purchased from Analtech. Unless otherwise noted, all NMR analyses were conducted in $CDCl_3$, on a Varian VXR-300, and were referenced to chloroform at ∂ 7.27. IR spectra were recorded on a Digilab FTS-40 or Perkin-Elmer 1610 FTIR. MS were obtained on a VG 7070E-HF or Reibermag R-10-10-C. Elemental analyses were within ±0.4% as determined by Desert Analytics, Tucson, AZ.

2,5,5-Trimethyl-2-[2'-[4''-(1(S)-carboxy-2-$^2H_3$-ethyl)-1''(R)-methyl-3''-[(trimethylsilyl)methylene]cyclohex-2''-yl]ethyl]-1,3-dioxane (5).

To an ice cold solution of diisopropylamine (1.15 ml, 8.2 mmol) in dry THF (5 ml) under N_2 was added n- BuLi (3.42 ml of 2.5 M solution in hexane, 8.5 mmol). The mixture was stirred at 0°C for 15 min. and then cooled to - 78°C. The acid **4** (1.5 g, 3.78 mmol) in dry THF (7 ml) was added and the mixture was allowed to come to room temp over 30 min., then heated at 50°C for 2 hrs, recooled to 0°C, and iodomethane-d_3 (590 μl) was added. The mixture was stirred at room temp for 1 hr, poured onto ice cold saturated aq. NH_4Cl solution, extracted with EtOAc, washed with water, dried ($MgSO_4$) and evaporated in vacuo to give 1.46 g (93%) of the product as viscous liquid. ^1H NMR δ 5.3 (s, 1H), 3.52 (d, 2H), 3.42 (dd, 2H), 2.78 (m, 1H), 2.38 (m, 1H), 2.12 (m, 1H), 1.82 (m,

5H), 1.48- 1.70 (m, 4H), 1.34 (s, 3H), 0.11 (s, 9H). IR: 3400-2400, 1735, 1704, 1598, 1470, 1273, 1258, 1088, 1020, 867, 834 cm^{-1}. ^2H NMR (on crude sample before purification) δ 3.3 (bs, rel. int. 1H), 2.53 (bs, rel.int. 4.3H), 1.00 (bs, rel. int. 28.3H).

Artemisinin-d_3 (1).

To a solution of 9-CD_3 ketal acid **5** (1.02 g, 2.46 mmol) in dry CH_2Cl_2 (235 ml) at -78°C was bubbled a stream of O_3/O_2 (4 P.S.I., 0.04 l/min., 80 V) for 10 min. until the reaction mixture turned blue. The reaction was then purged with O_2 and then with N_2. To the mixture was added silica gel (11.8 g), 15% aq. H_2SO_4 (1 ml) allowed to come to room temp and stirred for 3 days. The solids were filtered and washed with CH_2Cl_2 and EtOAc. The filtrate was washed with satd. aq. $NaHCO_3$ solution, dried ($MgSO_4$) and evaporated to give the crude product which was purified by flash chromatography. Elution with 20% EtOAc/ hexane gave 225 mg (32%) of pure product. mp. 155-157°C. ^1H NMR: δ 5.86 (s, 1H), 3.38 (d, 1H, J = 5.1 Hz), 2.38-2.47 (m, 1H), 1.74- 2.08 (m, 5H), 1.45 (s, 3H), 1.38-1.44 (m, 3H), 1.08 (m, 2H), 0.99 (d, 3H, J = 5.8 Hz). IR: 2952, 2928, 2909, 2848, 1735, 1452, 1188, 1130, 1112, 1003, 882 cm^{-1}. DCIMS-NH_3: 303 ($M+NH_4$), 286 (M+H), 240, 222, 212, 195, 182, 166, 154, 138. Anal. for $C_{15}H_{19}O_5D_3$: Calc: C, 63.12; H, 7.78. Found: C, 63.12; H, 7.61. ^2H NMR δ 1.08 (bs).

Dihydroartemisinin-d_3 (2).

Artemisinin-d_3 **1** (400 mg, 1.4 mmol) in CH_2Cl_2 (40 ml) was cooled to -78 C. To this solution was added (5.4 mmol) of DIBAL_H (x M in Hexanes). The solution was stirred at -78 C for 2 hrs. The reaction mixture was quenched with 30 % AcOH/ MeOH and evaporated to dryness under reduced pressure. The white residue was extracted with EtOAc. The EtOAc extracts were combined, filtered and evaporated to dryness to give white solid which was crystallised in EtOAc/ hexane to give 316 mg (75%) of pure product as white solid. mp blah ^1H NMR: δ 5.60 (s, 1H), 5.39 (s, 1H), 5.29 (m, 1H), 4.76 (m, 1H), 2.65 (m, 1H), 2.30-2.42 (m, 3H), 1.96-2.11 (m, 3H), 1.45-1.94 (m, 11H), 1.44 (s, 3H), 1.43 (s, 3H) 1.18-1.40 (m, 5H), 0.97 (m, 1H), 0.96 (d, 6H, J = 6.2 Hz). IR: 3375, 2944, 2922, 2850, 1572, 1445, 1375, 1173, 1091, 1010, 958, 882, 846 cm^{-1}. DCIMS-NH_3: 305 ($M+NH_4$), 287 (M+H), 270, 252, 237, 224, 198, 180, 155, 137. Anal. for $C_{15}H_{21}O_5D_3$: Calc: C, 62.68; H, 8.42. Found: C, 62.92; H, 8.21. ^2H NMR δ 0.96 (bs).

Arteether-d_3 (3).

Dihydroartemisinin-d_3 **2** (316 mg, 1.10 mmol) was dissolved in dry ether (45 ml). To the solution was added absolute EtOH (300 µl), followed by $BF_3 \cdot OEt_2$ (158 µl). The reaction mixture was stirred at room temp for 24 hrs., washed successively with 5% aq. $NaHCO_3$ and H_2O, dried ($MgSO_4$) and evaporated to dryness under reduced pressure. The resultant crude mixture was purified by flash chromatography. Elution with 15% EtOAc/ hexane gave 260 mg (75%) of pure product which was crystallized in hexane. mp 79-81°C. ^1H NMR δ 5.39 (s, 1H), 4.78 (d, 1H, J = 3.6 Hz), 3.85 (AB, 1H, J = 7.1, 7.1, 7.1, 9.8 Hz), 3.48 (AB, 1H, J = 7.1, 7.1, 7.1, 9.8 Hz), 2.57 (m, 1H), 2.38 (ddd, 1H, J = 4.0, 13.4, 14.5 Hz), 2.05 (dd, 1H, J = 2.9, 4.8 Hz), 2.00 (dd, 1H, J = 3.1, 4.8 Hz) 1.46-1.95 (m, 7H), 1.43 (s, 3H), 1.24 (dd, 1H, J = 6.4, 11.4 Hz) 1.17 (t, 3H, J = 6.2 Hz), 0.93 (d, 3H, J= 7.5 Hz). IR: 2969, 2951, 2936, 2915, 2878, 2848, 2227, 2208, 1459, 1433, 1374, 1341, 1249, 1224, 1205, 1195, 1124, 1096, 1043, 1018, 992, 964 cm^{-1}. DCIMS-NH_3: 333 ($M+NH_4$), 316 (M+H), 298, 270, 252, 237, 224, 184, 166, 138, 124. Anal. for $C_{17}H_{25}O_5D_3$: Calc: C, 64.72; H, 8.95. Found: C, 65.01; H, 9.04. ^2H NMR δ 0.88 (bs).

Acknowledgements

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