Radiolabeled Antimalarials: Synthesis of ^{14}C-Artemisinin

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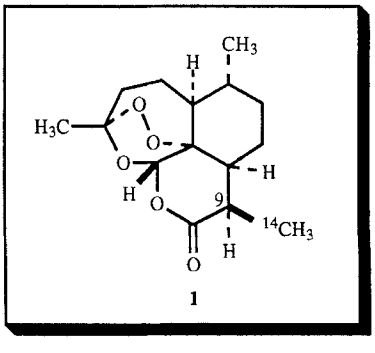
Summary

The stereoselective total synthesis of the antimalarial agent ^{14}C-(+)-artemisinin (**1**), incorporating ^{14}C at C-16 (C-9 methyl) is reported, in 18% radiochemical yield from acid **3**.

Key Words: malaria, artemisinin, radiolabeled, carbon-14

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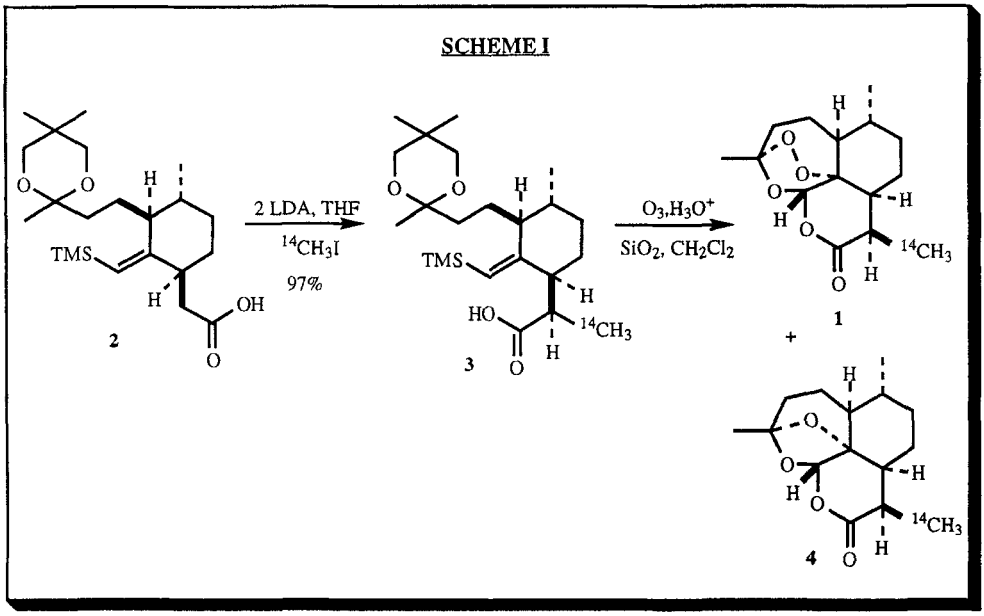
The subsequent evolution of artemisinin-type compounds into a therapeutically useful regimen has offered partial solutions to such problems as poor oral availability, water solubility, and short plasma half-life, resulting in numerous analogs (8-11). Studies devoted to drug stability and metabolism should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

Results and Discussion

The ease of construction of the compound was enhanced by the fact that we had access to a large amount of a total synthesis intermediate, ketal acid **2** (12) (Scheme I). Alkylation of **2** with $^{14}CH_3I$ gave acid **3** as a single stereoisomer in 92% yield. Ozonolysis of the crude mixture followed by acid catalyzed cyclization afforded ^{14}C-artemisinin **1** as well as a small amount of slightly less polar byproduct, which was judged to be the deoxy compound **4** based on comparative TLC and similar behavior observed during cyclization of the natural product (12).



In short, we report the stereoselective synthesis of ^{14}-C-(+)-artemisinin bearing ^{14}C at C-16 (or C-9 methyl), a metabolically stable position. The labeled natural product had a specific activity of 7.26 mCi/mmol and was 98.3% radiochemically pure (99.9% chemical purity). 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. ^{14}C-artemisinin has been used to gain access to other radiolabeled analogs now under clinical investigation; these results will be published separately.

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 Baker "flash" silica gel. ^{14}C-methyl iodide was obtained from Du Pont New England Nuclear Research Products. Autoradiographic analysis was performed by eluting the sample on an analytical TLC plate, using appropriate visualization (X-ray and/or H_2SO_4), and scraping the plate in fractions; the % radiopurity was calculated by dividing the mCi of the product fraction by the total mCi on the plate.

2,5,5-Trimethyl-2-(2'-(4"-(1"'-carboxy-2"-14C-ethyl)-1"(R)-methyl-3"-[(trimethylsilyl) methylene]cyclohex-2"-yl)ethyl)-1,3-dioxane (3).

A solution of diisopropylamine (2.2 mmol) in dry THF (4 ml) was cooled to 0°C and treated with n- BuLi (1.42 ml of 1.55 M solution in hexane, 2.2 mmol). The resulting solution was stirred at 0°C for 15 min. and then cooled to - 78°C. The acid **2** (396 mg, 1.0 mmol) in dry THF (2 ml) was added slowly. The resulting solution was allowed to warm to room temperature, then heated at 50°C for 2 hrs. The resulting solution was freeze-degassed and carbon-14 methyl iodide (350 mg, 2.44 mmol., 117 mCi, 48 mCi/mmol) was added by vacuum transfer at -196°C. The resulting solution was stirred under a closed system at the vapor pressure of THF at room temperature for 1 hr. Next, the excess $^{14}CH_3I$ and THF solvent were removed by vacuum transfer for waste removal. The dry residue of crude product was treated with saturated ammonium chloride (10 mL) and extracted with chloroform (4 x 10 mL). The lower organic phases were washed in succession with saturated sodium chloride, and concentrated. The crude product was purified with a column of silica gel eluting with a varying concentration of hexane/(1% acetic acid in ethyl acetate) from 80/20 to 60/40. Evaporation left 377 mg of ^{14}C-**3** (42.3 mCi, 92% yield).

^{14}C-Artemisinin (1).

Ozone (7 psi, 0.4 L/min, 70 V) was bubbled into a solution of **3** (.92 mmol) in CH_2Cl_2 (100 mL) at -78°C for 4 min. The excess ozone was purged with argon bubbling and the solution was treated with 35 mg 2,6-Di-*tert*-butyl-4-methylphenol (BHT) in CH_2Cl_2 (1 mL). Next silica gel (10 g) was added followed by 3M H_2SO_4 (3.5 mL). The resulting mixture was allowed to warm to room temperature and stir for 16 hr. The mixture was then treated with sodium bicarbonate (3 g) and stirred for 1 hr. The crude product was isolated by filtration, rinsing with CH_2Cl_2/EtOAc (90/10) (100 mL). The solvent was removed *in vacuo* and the crude product was placed on a column of silica gel (32 g) and eluted with a varying concentration of hexane/ethyl acetate (95/5) to (90/10). After elution with 200 mL of 95/5 and 400 mL 90/10, 2.5 mCi of deoxy compound **4** was collected from 50 mL of 90/10. The next 20 mL of 90/10 gave 0.2 mCi of a mixture of **1** and **4**, and the next 100 mL of 90/10 gave 7.8 mCi of product **1**. Analysis by autoradiography revealed product **1** to be 95.3% radiopure. This was repurified by the above procedure, providing **1** with 98.3% radiochemical purity. Dilution of this product with natural product artemisinin provided 300 mg of **1** with a specific activity of 7.26 mCi/mmol. The radiochemical purity remained at 98.3% while the chemical purity was >99.9%. DCIMS-NH_3: m/z 302 ($M+NH_4$), 285 ($M+H^+$), 267, 239, 211.

Acknowledgements

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