A Regiospecifically Oxygen-18 Labeled 1,2,4-Trioxane: A Simple Chemical Model System To Probe the Mechanism(s) for the Antimalarial Activity of Artemisinin (Qinghaosu)

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Among infectious diseases, malaria is the most widespread. Worldwide, about 300 million people are infected, and 1-2 million die each year.[1] As malarial parasite resistance to alkaloids such as quinine has increased, new non-alkaloidal antimalarial drugs such as the 1,2,4-trioxane artemisinin (qinghaosu, **1**) have become increasingly important.[2] Current understanding invokes hemin-catalyzed reduction of the trioxane unit in artemisinin as the key step activating it into one or more cytotoxic compounds that kill malarial parasites.[3] The hemin-rich internal environment of malarial parasites is thought to be responsible for the selective toxicity of trioxanes like artemisinin toward these parasites.[4] To gain a better understanding at the molecular level of this antimalarial activity of activated artemisinin, we report here the preparation and cleavage reactions of simpler, regiospecifically oxygen-18 labeled 1,2,4-trioxane **5c** that also is a potent antimalarial compound.[5]

Oxygen-18 was introduced from $^{18}OH_2$ during the conversion of nitrile **2** into methyl ketone **3** (Scheme I).[5,6] 1,2-Dioxetane formation, using either singlet molecular oxygen or triethylsilyl hydrotrioxide,[7] gave intermediate dioxetane **4** that rearranged under the influence of (*tert*-butyldimethylsilyl) triflate into 1,2,4-trioxane **5a**.[7] Fluoride-induced desilylation produced trioxane alcohol **5b**. The location of ^{18}O at position 4 in the 50% ^{18}O-enriched 1,2,4-trioxane **5b** was established by ^{13}C NMR spectroscopy, in comparison with an unlabeled version of trioxane **5b**, showing doublets at 105.3 and 100.2 ppm characteristic of carbon atoms at positions 3 and 5 (Scheme I);[6] only carbons 3 and 5 showed one-bond ^{18}O-induced isotope shifts. This isotopic labeling experiment supports the proposed zwitterionic peroxide pathway (see arrows in structure **4**) for rearrangement of keto dioxetanes such as **4** into 1,2,4-trioxanes such as **5a**.[8] Trioxane alcohol **5b** was treated with tosyl chloride and triethylamine to form trioxane tosylate **5c**.[5] Tosylate **5c** was chosen for reduction experiments because it is an active antimalarial agent[5] and because oxyanionic intermediates formed during the reduction process were expected to be trapped intramolecularly by displacement of the tosylate group.

Trioxane tosylate **5c** was treated separately with several established reducing agents. Samarium diiodide (at 25 °C),[9] zinc (at 25 °C),[10] Ph_3CLi (at -78 °C),[11a] and Bu_3SnH/AIBN (in refluxing benzene)[12] all produced 1,3-dioxolane tosylate **8** within 4 h in good to excellent yields. ^{13}C NMR spectroscopy confirmed that ^{18}O was located exclusively at the exocyclic hemiacetal position.[6] No reaction occurred when trioxane **5c** was treated with typical peroxide-cleaving reagents such as Me_2S, Ph_3P, or sodium dithionite.[11b] The mechanism in Scheme II is proposed to account for the generation of dioxolane **8**.[13] Electron transfer to trioxane **5c** gives a radical anion; specifically, radical anion **6** unzips with extrusion of methoxide anion to form trioxane ring-cleaved intermediate **7** that rezips to produce the observed dioxolane **8**. Formation of a 1,3-dioxolane by the deoxygenation of a 1,2,4-trioxane has physiological relevance because malarial parasites operate on trioxanes like artemisinin to produce the corresponding dioxolane.[14] Had radical anion **6** been formed with the opposite arrangement of radical and anionic centers, then methoxy radical extrusion and rezipping would have led ultimately to intramolecular oxyanion displacement of the pendant tosylate group to form cyclic ether **9**.[15] Indeed, cyclic ether **9** was produced in excellent yield when hydroxy tosylate **8** was treated with the base sodium hydride and also when trioxane **5c** was treated at 0-25 °C with dimethylcopperlithium, a basic reducing agent.[16] Also, treating the trioxane iodide analogous to trioxane tosylate **5c** with dimethylcopperlithium gave cyclic ether **9** directly and exclusively.[13]

Trioxane tosylate **5c** was treated at room temperature in THF also with two different sources of ferrous ions to simulate the biologically important erythrocyte hemin-catalyzed cleavage of the trioxane unit in artemisinin.[3] Although ferrous bromide and hemin/$PhCH_2SH$ (no reaction occurred without $PhCH_2SH$)[3] both induced rupture of the peroxide linkage in trioxane **5c**, products **11**, **12**, and **14** (different from dioxolane **8**) were produced.[13] Ring-contracted tetrahydrofuran acetal **11**, aldehyde **12**, and hydroxy dioxolane **14** are typical of the metabolites formed from trioxanes such as artemisinin in the presence of rat liver microsomes;[14] indeed, when we treated artemisinin with ferrous bromide, an acetal like **11** and a hydroxy dioxolane like **14** were formed along with a dioxolane like **8**. When hemin/$PhCH_2SH$ was used on trioxane **5c**, $PhCH_2SSCH_2Ph$ was formed, analogous to the protein thiol oxidation products (i.e., disulfides) observed when artemisinin itself and hemin interact in the presence of red cell membranes.[17] The radical mechanism in Scheme II, similar to the mechanism proposed earlier for the very high temperature

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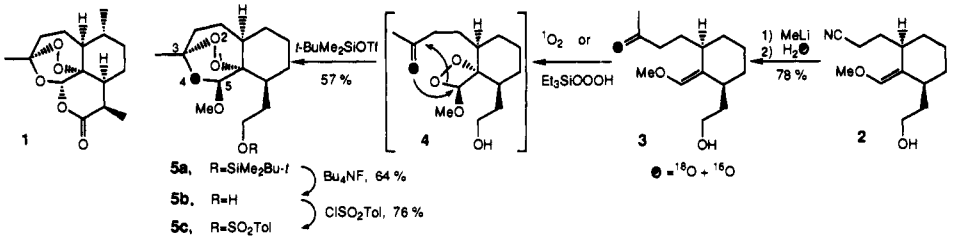
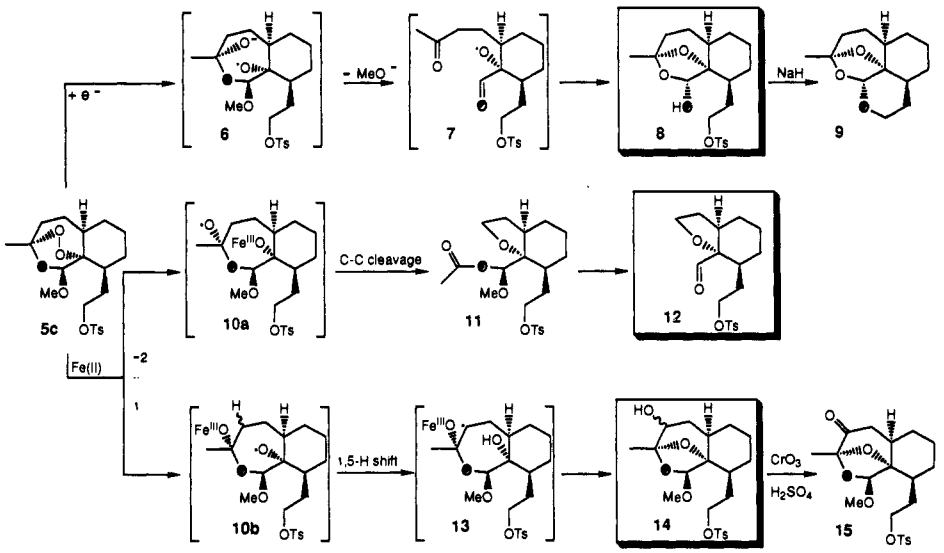
Scheme I



Scheme II



(190 °C) pyrolysis of artemisinin,[18] is offered to account for these room-temperature results. Iron(II)-induced cleavage of the peroxide bond in trioxane **5c** leads to radical intermediates **10a** and **10b** in about a 2:1 ratio: C–C bond cleavage of **10a** initially produces labile ring-contracted tetrahydrofuran acetal **11** (characterized by ^1H and ^{13}C NMR) with ^{18}O located in the acetoxy group as shown in Scheme II (mass spectrum, M – $CH_3CO^{18}O$) and then produces stable electrophilic tetrahydrofuran aldehyde **12** lacking ^{18}O. 1,5-Hydrogen atom abstraction in radical intermediate **10b** ultimately leads to stable dioxolane alcohol **14** as a mixture of two diastereomers with ^{18}O not located in the methoxyl group (mass spectrum M – CH_3O). Subsequent oxidation of this isomeric mixture of alcohols **14** gave the corresponding dioxolane ketone **15** as a single product.[13] The overall yields of isolated aldehyde **12** and hydroxy dioxolane **14** ranged from 60 to 70%.

In summary, these reactions of trioxane **5c** for the first time **(1) provide firm mechanistic evidence that deoxygenation of a 1,2,4-trioxane into the corresponding 1,3-dioxolane occurs via a tandem unzipping–zipping process and (2) show that trioxane cleavage by ferrous ions follows a different mechanistic course and leads to different products than trioxane cleavage by nonferrous reducing agents.** These results may help the development of better antimalarial trioxanes.[19,20]

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Supplementary Material Available: Listing of full experimental details and spectral data for compounds **3**, **5a–c**, **8**, **9**, **11**, **12**, **14**, and **15** (36 pages). Ordering information is given on any current masthead page.

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Remarkable Regioselectivity in the Chemical Glycosylation of Glycal Acceptors: A Concise Solution to the Synthesis of Sialyl-Lewis X Glycal

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The cell-surface-bound polysaccharide sialyl-Lewis X antigen (SLe^x, **1**)[2] has recently been identified as a ligand for binding to the cell-adhesion molecules ELAM-1 and CD-62.[3] These proteins are expressed on cell membranes in response to tissue injury, and

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