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SYNTHESIS AND ANTIMALARIAL EVALUATION OF 2,3,5-TRIOXABICYCLO[2.2.2]OCTANES, MODELS FOR THE PUTATIVE PHARMACOPHORE OF QINGHAOSU (ARTEMISININ)

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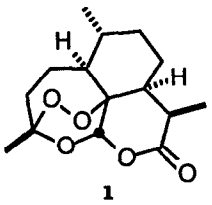
Abstract: 5-Hydroperoxy-2-methoxytetrahydropyrans are cyclized with transacetalization in the presence of Amberlyst-15 and molecular sieves to 2,3,5-trioxabicyclo[2.2.2]octanes, models of the proposed pharmacophore of qinghaosu. These bridged bicyclic trioxanes exhibit only marginal antimalarial activity.

The antimalarial drug qinghaosu (**1**) has initiated interest in the chemistry and biological activity of related peroxy compounds.[1-4] The unique 1,2,4-trioxane system embedded in the framework of qinghaosu has been proposed as the structural feature necessary for antimalarial activity.[5] A number of synthetic 1,2,4-trioxanes have been prepared and evaluated for activity in order to test this hypothesis.[6-10]

Many of the methods to prepare synthetic trioxanes have focused on the use of singlet oxygen[11,12] or its equivalent[8] to form the peroxy linkage. Alternatively, the use of ozonolysis, either to incorporate the hydroperoxide[13] or for the preparation of precursors in a cationic ozonide rearrangement has also been successful.[14] We have chosen to pursue a conceptually simple approach that involves the synthesis of suitably substituted secondary hydroperoxides and subsequent cyclization to trioxanes.



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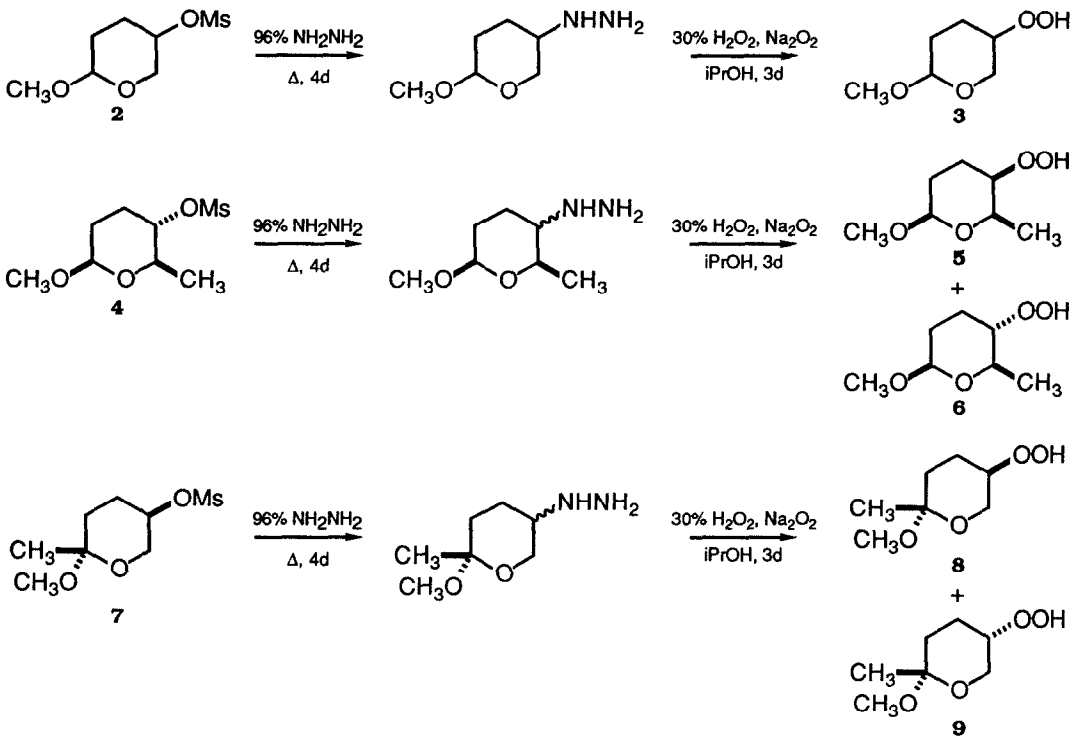
We wish to report the synthesis of four 'stripped-down' bridged bicyclic trioxanes, 2,3,5-trioxabicyclo[2.2.2]octane, exo- and endo-6-methyl-2,3,5-trioxabicyclo[2.2.2]octane, and 4-methyl-2,3,5-trioxabicyclo[2.2.2]octane from the corresponding 5-hydroperoxy-2-methoxytetrahydropyrans. These structures, while mimicking the bridged trioxane of qinghaosu, contain little other functionality. Through synthesis and antimalarial testing, we intend to clarify whether the bridged trioxane is not only necessary but also sufficient to impart antimalarial activity.

We have recently described a method for the synthesis of secondary hydroperoxides from the corresponding alcohols by way of the mesylate esters.[15] As an example, reaction of mesylate **2** with anhydrous hydrazine at reflux for four days followed by treatment of the resulting crude product with 30% hydrogen peroxide and Na_2O_2 in 2-propanol provided **3** as a mixture of cis and trans isomers in 30% yield.[16] Using the same sequence of steps, **5** and **6** were prepared from **4** in 64% yield in a 1:1 ratio. Mesylate **7** gave the hydroperoxides **8** and **9** in a 1:1 ratio in 15% yield. Compounds **5** and **6** were separated by silica gel chromatography, as were **8** and **9**. The stereoisomeric pairs of hydroperoxides, **5** / **6** and **8** / **9**, were assigned based on the magnitude of the coupling constants in the ^1H NMR spectra (axial/equitorial differentiation), upon comparison with spectra of the corresponding mesylates (the relative configuration of **4** was assigned by X-ray), and on NOESY experiments.

Scheme 1

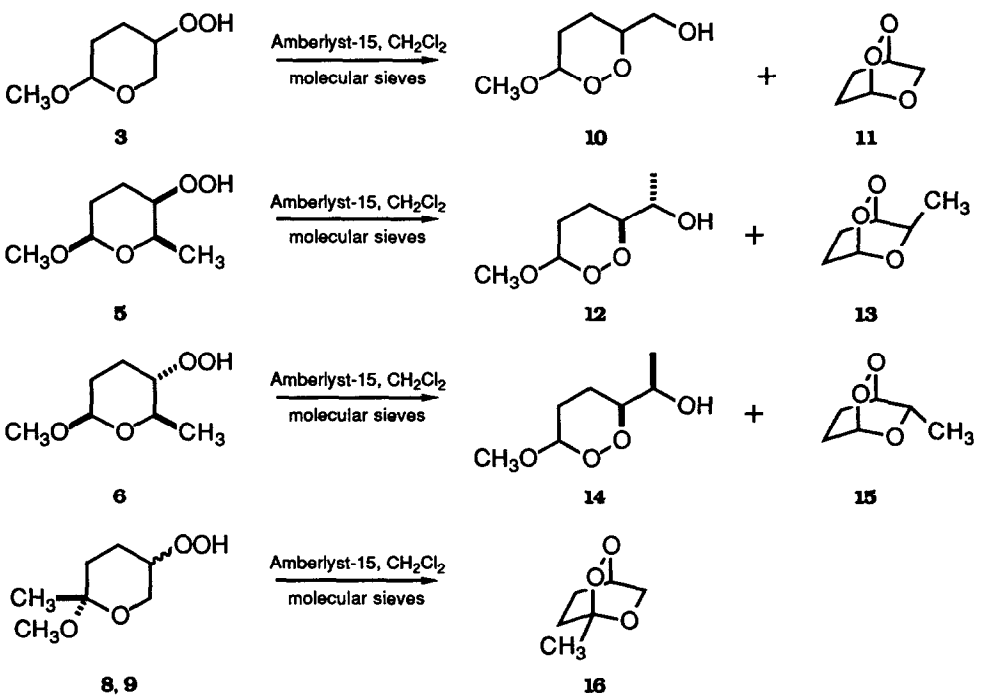


Treatment of hydroperoxy acetal **3** with Amberlyst-15 ion-exchange resin in dichloromethane at room temperature gave a transacetalization product (Scheme 2). Upon NMR characterization, it became clear that the methoxy group was still intact and no hydroperoxy proton was detected in the ^{1}H NMR spectrum. The structure was therefore assigned as the monocyclic peroxy acetal **10**. In order to form the bridged bicyclic compound, the reaction was carried out in the presence of 3Å or 4Å molecular sieves. Stirring at room temperature overnight gave a separable mixture of 52% of **10** and 18% of **11**.[16,17]

Under the same conditions (Amberlyst-15 and molecular sieves), hydroperoxides **5** and **6** gave cyclized materials. Compound **5** gave monocyclic and bicyclic peroxides **12** and **13**, separable by chromatography, in 25% and 21% yields, respectively.[18] Dioxane **12** is a mixture of diastereomers at the acetal carbon. On the other hand, **6** provided isolable quantities of only the bicyclic peroxide **15** (13%) even though a small amount of **14** could be detected by TLC.[19]

In constrast to the low yields observed for the cyclizations of **3**, **5**, and **6**, the reaction of a 1:1 mixture of **8** and **9** in the presence of Amberlyst-15 and molecular sieves provided a 71% yield of trioxane **16**, a quantitative yield based on unrecovered starting material.[20] These results suggest that formation of bicyclic ketal trioxanes proceeds with greater ease and/or the product trioxanes are more stable than in the related acetal systems.

Scheme 2



Compounds **3**, **5**, **6**, **8** -**13**, **15**, and **16** were screened for biological activity in both chloroquine-resistant (W-2) and chloroquine-sensitive strains (D-8) of human falciparum malaria(Table).[21] The hydroperoxides were only marginally active, possessing IC_{50}'s ranging from 472-22,000 ng/mL in D-8. More significantly, the trioxanes tested were also only marginally active, with IC_{50}'s generally >5000 ng/mL. In comparison, qinghaosu has an IC_{50} of 2.02 ng/mL. Although the range of IC_{50} values is quite large for some of the compounds, the clear message is that these trioxanes are either insufficient structurally or are lacking some necessary physico-chemical property. Among the factors that we expect to play a role are the water solubility and the volatility of the analogues. For example, the trioxanes are volatile under water aspirator pressure at room temperature.

Table

Compound	Indochina W-2 IC_{50} ng/mL	Sierra Leone D-8 IC_{50} ng/mL	Compound	Indochina W-2 IC_{50} ng/mL	Sierra Leone D-8 IC_{50} ng/mL
3	1046	472	12	12276	7354
5	8000-18088	9569-21648	13	13098	8460
6	3436	1006	15	28849	28533
8, 9	7079-48759	10000-46608	16	>100000	50000
10	454	531	qinghaosu	1.09	2.02
11	60-50000	979-50000	chloroquine	50.03	2.12

This very simple approach to trioxanes opens possibilities for the design and preparation of new, carefully tailored antimalarial compounds without the synthetic constraints of using photooxygenation for the incorporation of the peroxy group. We are exploring the synthesis of a number of substituted bridged bicyclic trioxanes for further structure-activity relationship studies.

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(16) All compounds prepared gave satisfactory ^{1}H, ^{13}C NMR data and elemental analysis (± 0.40%) or HRMS (CI).
(17) ^{1}H NMR for **10** (360 MHz, $CDCl_3$, all signals are doubled): 1.5-2.1 (m, 4H), 2.15 (br, 1H, OH), 3.47, 3.50 (s, 3H, OMe), 3.62-3.85 (m, 1H), 4.15-4.34 (m, 1H, 4.81 (m, 1H). ^{13}C NMR for **10** (90 MHz, $CDCl_3$): 20.29, 21.42, 25.67, 26.75, 55.67, 56.69, 63.46, 64.53, 81.63, 82.33, 102.37, 105.08. ^{1}H NMR for **11** (360 MHz, $CDCl_3$): 1.97 (m, 1H), 2.18-2.27 (m, 2H), 2.39 (m, 1H), 4.01 (dd, 1H, *J*=1.8, 9.7 Hz), 4.23 (dt, 1H, *J*=2.5,9.6 Hz), 4.52 (ddd, 1H, *J*=9.7, 2.5, 2.5 Hz), 5.20 (dd, 1H, *J*=3, 4.9 Hz). ^{13}C NMR for **11** (90 MHz, $CDCl_3$): 23.08, 26.16, 67.48, 72.34, 95.47.
(18) ^{1}H NMR for **12** (360 MHz, $CDCl_3$, all signals are doubled): 1.17 (d, 6H, Me), 2.04-1.3 (m, 8H), 2.36 (s, lH, OH), 2.56 (s, lH, OH), 3.46 (s, 3H, OMe), 3.49 (s, 3H, OMe), 3.72 (m, 2H, MeCHOH), 3.96 (m, 2H, OOCH), 4.81 (m, 2H, peroxyacetal). ^{13}C NMR for **12** (90 MHz, $CDCl_3$): 17.84 (2 carbons). 20.52, 21.29, 25.29, 26.75, 55.33, 56.14, 67.77, 68.54, 85.39. 85.60,101.89, 104.55. ^{1}H NMR for **13** (360 MHz, $CDCl_3$): 1.52 (d, 3H, *J*=6.4 Hz, Me), 1.92 (m, lH), 2.10 (m, lH), 2.23 (m, lH), 2.41 (m, lH), 3.94 (ddd, lH, *J*=1.5, 1.4, 1.6 Hz, OOCH), 4.09 (dq, lH, *J*=l, 6.4 Hz, MeCHO), 5.19 (dd, lH, *J*=2.5, 6 Hz, peroxyacetal). ^{13}C NMR for **13** (90 MHz, $CDCl_3$): 19.80, 23.38, 25.24, 72.16, 74.61, 95.13.
(19) ^{1}H NMR of **15** (360 MHz, $CDCl_3$): 1.26 (d, 3H, *J*=6.6 Hz, Me), 2.26-2.04 (m, 4H), 4.00 (m, lH, OOCH), 4.66 (q, lH, *J*=6.6 Hz, OCHMe), 5.20 (m, lH, peroxyacetal). ^{13}C NMR of **15** (90 MHz, $CDCl_3$): 17.85, 18.72, 25.93, 71.73, 76.24, 95.87.
(20) ^{1}H NMR for **16** (360 MHz, $CDCl_3$): 1.30 (s, 3H, Me), 1.94 (m, 1H), 2.16 (m, 2H), 2.34 (m, 1H), 4.00 (dd, 1H, *J*=1.7, 9.5 Hz, OCH endo), 4.23 (m, 1H, OOCH); 4.49 (ddd, 1H, *J*=2.4, 2.4, 9.6 Hz, OCH exo). ^{13}C NMR for **16** (90 MHz, $CDCl_3$): 23.33, 23.72, 30.31, 67.98, 71.54, 99.52.
(21) The intrinsic antimalarial activity of each compound and a simultaneous qinghaosu control were determined using modifications of a semiautomated microdilution technique[22,23] against cloned isolates[24] of human falciparum malaria. Drugs were dissolved in DMSO and then diluted with culture medium with 10% human plasma. Parasites were exposed to serial dilutions of drug in microtiter plates incubated at 37 °C in a microaerophilic environment for a period of 24 h prior to the addition of ^{3}H hypoxanthine which served as an index of exponential parasite growth. After an additional 18 h of incubation, particular matter were harvested from each well using an automated cell harvester. The amount of incorporation of radiolabelled hypoxanthine was determined by scintillation spectrophotometry. IC_{50}'s were calculated from concentration response data using non-linear regression.
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