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SYNTHESIS AND ANTIMALARIAL EVALUATION OF 2,3,5-TRIOXABICYCLO[2.2.3]NONANE, A MODEL FOR THE PUTATIVE PHARMACOPHORE OF ARTEMISININ

Dee Ann Casteel,*† S. Prasad Peri,†and Lucia Gerena‡
†Division of Medicinal and Natural Products Chemistry
College of Pharamcy, University of Iowa, Iowa City, IA 52242 and
‡Division of Experimental Therapeutics, Walter Reed Army Institute of Research,
Washington, DC 20307-5100

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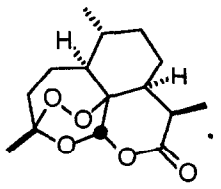
Abstract: The title compound was prepared from the known 6-tetrahydrooxepanol by formation of the 6-mesylate followed by treatment with acidic methanol to yield the methyl acetal. Reaction of 2-methoxy-6-mesyloxytetrahydrooxepane with anhydrous hydrazine followed by treatment with 30% hydrogen peroxide and sodium peroxide provided the diastereomeric mixture of 6-hydroperoxides. The hydroperoxides cyclized upon treatment with acid to give trioxabicyclo[2.2.3]bicyclononane. The bridged bicyclic trioxane exhibited only marginal antimalarial activity.

The antimalarial drug artemisinin (**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 **1** has been proposed as the structural feature necessary for antimalarial activity.[5] In our SAR studies of artemisinin, we are pursuing an approach that involves the synthesis of suitably substituted hydroperoxides and subsequent cyclization to trioxanes. Through synthesis and antimalarial testing, we intend to clarify whether the bridged trioxane is not only necessary but sufficient to impart antimalarial activity. Our earlier studies have shown that 2,3,5-trioxabicyclo[2.2.2]octanes are only marginally active as antimalarial agents.[6] We now report the synthesis of the analogous 2,3,5-trioxabicyclo[2.2.3]-nonane (**2**) and its activity against malaria parasites.



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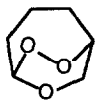
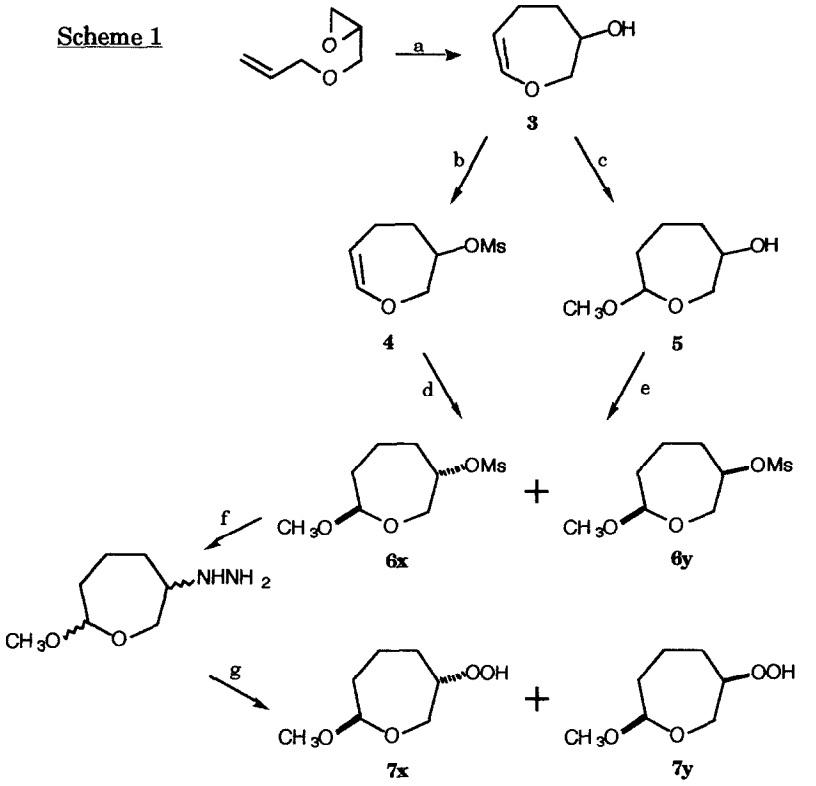


2

The synthesis of **2** begins with the known 6-tetrahydrooxepanol (**3**), prepared by cyclization of allyl glycidyl ether (Scheme 1).[7] Two routes were then explored for the preparation of the mesyl acetal (**6**). Compound **4** was formed from **3** using standard conditions and was then treated with methanol and acid to give acetal **6** in 67% yield. Alternatively, compound **3** could be treated with acid and methanol to give **5** (52%) followed by reaction with methanesulfonyl chloride to give **6** (20%).

With compound **6** in hand, the preparation of the corresponding hydroperoxides proceeded using our published protocol.[8] Mesylate **6** was reacted with anhydrous hydrazine at reflux for several days. After an extractive work-up, the residue was then treated with hydrogen peroxide and sodium peroxide in 2-propanol to provide hydroperoxide **7** which was purifed by chromatography in 54% overall yield from **6**.

Scheme 1



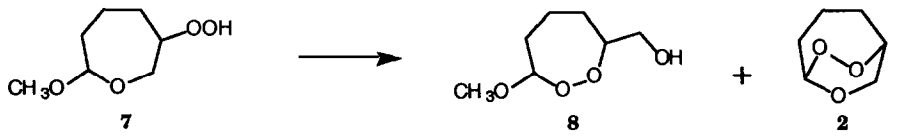
Reaction Conditions: (a) see ref 7; (b) MsCl, Et_3N, 76%; (c) MeOH, $Ph_3P{\cdot}HBr$, 52%; (d) MeOH, TsOH, 67%, (e) MsCl, Et3N, 20%; (f) anhydr NH_2NH_2, reflux, 4 d, 100% (mass balance); (g) H_2O_2, Na_2O_2, iPrOH, 3d, 54%.

Compounds **6** and **7** exist as mixtures of cis and trans isomers which can be separated by chromatography and characterized. Although ^1H NMR resonances were assigned by decoupling experiments on compounds **6x** and **6y**, it was not possible to identify the isomers using spectroscopic methods due to the conformational flexibility of the oxepane system. Our studies on the stereochemical issues in these systems will be published elsewhere.

The mixture of cis and trans hydroperoxides **7** was treated with acid to effect cyclization to the peroxy acetal compound Some selectivity in the course of the cyclization was observed depending on which acid was used A number of acids (formic acid, camphor sulfonic acid, Amberlyst 120) that were examined had no effect on **7** Methanesulfonic acid produced only dioxane **8** in a 40% yield. When Amberlyst-15 ion-exchange resin was used in the presence of molecular sieves, however, mixtures of **8** and the trioxane **2** were isolated in 25% and 21% yields, respectively. Trioxane **2** was isolated in quantitative yield upon reaction of **7** with perchloric acid in CH_2Cl_2.

Both isomers of the hydroperoxide **7** as well as trioxane **2** were screened against *P falciaprum* clones.[9] The results are shown in the Table along with the data for other active antimalarial agents As had been seen for other hydroperoxides, the activity in these tests is marginal at best The trioxane (**2**) shows better activity but not nearly enough to establish the 2,3,5-trioxabicyclo[2.2.3]nonane system as critical for antimalarial activity

Scheme 2



7 8 2

We are studying the preparation of water soluble analogues of **2** in order to determine whether physico-chemical properties of the trioxanes plays an important role in antimalarial activity.

Experimental

6-Methanesulfonyloxytetrahydrooxepane (**4**)
6-Tetrahydrooxepanol (**3**) (1.2 g, 11 mmol), Et_3N (2.7 mL, 20 mmol), and methanesulfonyl chloride (1.4 mL, 18 mmol) were combined in distilled CH_2Cl_2 (50 mL) and were allowed to stir for 30 min in a dry ice/acetone bath. The mixture was diluted with CH_2Cl_2 (50 mL) and then washed with 2% AcOH (3 x 25 mL). The organic layer was dried (Na_2SO_4) and evaporated under vacuum to give 2.6 g of a colorless oil. The product was purified by chromatography on silica gel using 20% ethyl acetate/hexanes as eluant to provide 1.2 g (76%) of the mesylate ^1H NMR: 2.20 (br m, 4H, C$\underline{H}_2$), 3.06 (s, 3H, OMs), 4.18 (q, 2H, OC$\underline{H}_2$), 4.81 (m, 1H, C$\underline{H}$OMs), 5.01 (m, 1H, OCH=C$\underline{H}$), 6.32 (d, 1H, OC$\underline{H}$=CH). ^{13}C NMR: 20.80, 31.84, 38.61, 73.60, 80.37, 108.96, 148.83.

2-Methoxy-6-methanesulfonyloxyoxepane (**6**)
6-Methanesulfonyloxytetrahydrooxepane (0.4 g, 2.08 mmol), triphenylphosphine hydrobromide (0.035 g, 0.1 mmol), and distilled dry CH_2Cl_2 (15 mL) were combined under nitrogen. Freshly distilled dry MeOH (0.25 mL, 6.24 mmol) was added via syringe and the reaction stirred for 5 h at RT. After dilution with CH_2Cl_2 (50 mL), the mixture was washed with brine (2 x 25 mL) and the organic layer was separated, dried (Na_2SO_4), and evaporated under vacuum to give a crude oil which was purified by silica gel chromatography to provide 0.3 g (67%) of the mixture of diastereomers.
Band 1: ^1H NMR: 1.44 (m, 1H), 1.63 (m, 3H), 2.06 (m, 1H), 2.30 (m, 1H), 3.02 (s, 3H, OMs), 3.35 (s, 3H, OMe), 3.69 (dt, 1H, OC$\underline{H}_2$), 3.89 (dd, 1H, OC$\underline{H}_2$), 4.54 (dd, 1H, C$\underline{H}$OMe), 4.66 (m, 1H. C$\underline{H}$OMs). ^{13}C NMR: 17.94, 33.92, 35.43, 38.45, 55.08, 62.94, 79.12, 102.77.
Bank 2: ^1H NMR: 1.58 (m, 2H), 1.73 (m, 2H), 2.19 (m, 2H), 3.08 (s, 3H, OMs), 3.36 (s, 3H, OMe), 3.80 (dt, 1H, OC$\underline{H}_2$), 4.01 (d, 1H, OC$\underline{H}_2$), 4.66 (dd, 1H, C$\underline{H}$OMe), 4.90 (d, 1H, C$\underline{H}$OMs). ^{13}C NMR: 17.43, 34.79, 35.63, 38.87, 55.11, 62.20, 79.66, 102.77.

2-Methoxy-6-hydrazinooxepane
2-Methoxy-6-methanesulfonyloxyoxepane (0.25 g, 1.1 mmol) was heated at reflux in anhydr. hydrazine (5 mL, 96%) for 4 d. The reaction mixture was washed with ether (3 x 20 mL) and the organic layers were washed with 50% aq KOH (5 mL). The ether layer was dried (Na_2SO_4) and evaporated under vacuum to give the crude hydrazino

Table

Compound	Indochina W-2[a] ng/mL			Sierra Leone D-8[a] ng/mL		
	IC_{50}	IC_{90}	IC_{10}	IC_{50}	IC_{90}	IC_{10}
7, isomer A	6524.88	12775.42	3332.49	7490.26	22824.73	2458.03
7, isomer B	15456.81	20933.22	11413.10	10053.20	38084.48	2653.75
2	...[b]	...[b]	...[b]	2449.32	4466.78	1343.06
artemisinin	0.72	1.05	0.49	0.83	2.13	0.32
chloroquine	70.02	93.12	52.65	2.69	3.61	2.01
mefloquine	2.09	3.73	1.17	6.86	38.47	1.22

[a] D-8 is a chloroquine-sensitive strain of *Plasmodium falciparum*; W-2 is chloroquine resistant.
[b] Not tested

derivative (0.17 g, quant.) which was used in the next reaction without purification.

2-Methoxy-6-hydroperoxyoxepane (**7**)
2-Methoxy-6-hydrazinooxepane (70 mg, 4 mmol) was dissolved in 2-PrOH (15 mL) and stirred with 30% aq H_2O_2 (10 mL). Na_2O_2 (50 mg, 0.6 mmol) was added and the reaction allowed to stir for 3 d The reaction mixture was diluted with CH_2Cl_2 (100 mL) and washed with brine (2 x 50 mL) The organic layer was dried (Na_2SO_4) and evaporated under vacuum to give a yellow oil. The crude hydroperoxides were purified on silica gel to give both diastereomeric hydroperoxides (54 %).
Band 1: ^{1}H NMR: 4.53 (q, 1H, C<u>H</u>-O-C), 4 06 (m, 1H, C<u>H</u>-OOH), 3.85 (d, 2H, C<u>H$_2$</u>-O-C), 3.4 (s, 3H, OC<u>H$_3$</u>), 2.10-2.0 (m, 2H, C<u>H$_2$</u>), 1.75-1.65 (m, 2H, C<u>H$_2$</u>), 1.4-1 25 (m, 2H, C<u>H$_2$</u>). ^{13}C NMR: 103.57, 83.67, 62 17, 55.04, 34.12, 31.94, 18,26. MS: 145 (M-OH), 131 1 (M-MeO).
Band 2· ^{1}H NMR: 8.44 (br s, 1H, OO<u>H</u>), 4.62 (q, 1H, C<u>H</u>-O-C), 4.13 (m, 1H, C<u>H</u>-OOH), 3.93 (d, 2H, C<u>H$_2$</u>-O-C), 3.36 (s, 3H, OC<u>H$_3$</u>), 2.09-2 03 (m, 2H, C<u>H$_2$</u>), 1.68-1.26 (m, 4H, C<u>H$_2$</u>) ^{13}C NMR: 102 87, 81 88, 59.79, 55.01, 34.79, 32.29, 17.85.

7-Hydroxymethyl-3-methoxydioxepane (**8**)
Compound **7** (10 mg, 0.06 mmol) was dissolved in freshly distilled CH_2Cl_2 (20 mL). Molecular sieves (4Å, 100 mg) and methanesulfonic acid (1 drop) were added. After stirring at room temperature, the reaction mixture was diluted with CH_2Cl_2 and washed with satd $NaHCO_3$. The organic layer was separated, dried (Na_2SO_4) and evaporated to give an oil which was purified by chromatography on silica gel to provide the dioxepane (4 mg, 40%) as a mixture of diastereomers (~6:1). ^{1}H NMR: 1.39-2.14 (m, 6H), 3 46 (s, 3H, OMe), 3.50 (m, 2H, C<u>H$_2$</u>OH), 4.32 (m. 1H, C<u>H</u>OOC), 4.74 (q, 1H, OC<u>H</u>O). ^{13}C NMR· 19.71, 20 93, 30 68, 32.16, 33 54, 34 70, 53.41, 55.62, 62.97, 63 55, 84 19, 87.49, 108.71.

2,3,5-Trioxobicyclo[2.2.3]nonane (**2**)
A mixture of the diastereomers of 2-methoxy-6-hydroperoxyoxepanes (50 mg, 0.3 mmol) dissolved in dry CH_2Cl_2 (25 mL) with molecular sieves (500 mg) was stirred with 2 drops of conc. $HClO_4$. After 30 min, when by TLC there was no starting material, the reaction mixture was filtered and diluted with CH_2Cl_2 (50 mL) It was then washed with satd. $NaHCO_3$ (2 x 20 mL) and dried with Na_2SO_4. Evaporation of the CH_2Cl_2 under vacuum gave the product as a white solid, mp 160-161 °C (40 mg, 100%). ^{1}H NMR: 5 13 (br s, 1H, acetal), 4.35 (t, 1H, C<u>H</u>-OOC), 3.95 (d, 1H, endo to endoperoxide), 3.52 (q, 1H, exo to endoperoxide), 1 87 (br s, 2H, C<u>H$_2$</u>), 1 58 (br s, 2H, C<u>H$_2$</u>), 1 35 (br s, 2H, C<u>H$_2$</u>). ^{13}C NMR· 103.80, 79 21, 69 13, 31.71, 28 95, 19.23. MS. 131 1 (M+1, 18%), 85 1 (M-46, 100%)

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(9) See ref 6 for details of the screening experiments.