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Photo-Oxygenation of Geraniol: Synthesis of a Novel Series of Hydroxy-Functionalized Anti-Malarial 1,2,4-Trioxanes†

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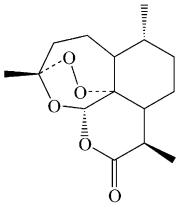
Abstract—Photo-oxygenation of geraniol **2**, an abundantly available allylic alcohol, furnished a mixture of mono- and di-hydroperoxy products; the latter have been used for the preparation of a novel series of hydroxy-functionalized anti-malarial 1,2,4-trioxanes (**7a–d, 8a–d**).

Artemisinin **1**, the active principle of the Chinese traditional drug against malaria, *Artemisia annua*, and its semi-synthetic derivatives are highly potent anti-malarials and are currently being used clinically to treat multidrug resistant malaria.[1] The peroxide group present in the form of a 1,2,4-trioxane is essential for the anti-malarial activity of these compounds. Currently, however, because of the limited availability of artemisinin and consequently its derivatives, focus is on the synthesis and anti-malarial activity of structurally simple 1,2,4-trioxanes.[2,3]



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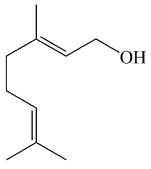

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Working toward these lines our laboratory has earlier reported a convenient and novel photo-oxygenation route for the synthesis of 1,2,4-trioxanes. The key step of this procedure is the preparation of β-hydroxy-hydroperoxides by photo-oxygenation of allylic alcohols and their subsequent condensation with aldehydes/ketones.[2c] Several of the trioxanes prepared using this method have shown excellent anti-malarial activity, both in vitro and in vivo.[2c,3b] In continuation with these studies, we have explored the potential of geraniol, an abundantly available monoterpene allylic alcohol, as a starting material for the synthesis of a novel series of 1,2,4-trioxanes.

Photo-oxygenation of geraniol[4] in CH_3CN at −10 to 0 °C furnished a mixture of hydroperoxides **3a**, **4a**, **5a** and **6a**, which on reduction with $NaBH_4$ in methanol furnished the mixture of respective alcohols **3b**, **4b**, **5b** and **6b**. This mixture on column chromatography on silica gel furnished two broad fractions containing diols **3b** and **4b** (38% yield), and triols **5b** and **6b** (10% yield). Acetylation of these fractions followed by column chromatography furnished acetyl derivatives **3c**, **4c**, **5c**, and **6c**, which were fully characterized by NMR and MS. Triacetate **5c**, though homogeneous by TLC and ^1H NMR data, its ^{13}C NMR data showed it to be a 1:1 mixture of two diastereomers.[11] Thus its precursors hydroperoxide **5a** and triol **5b** are also diastereomeric mixtures.

For preparation of trioxanes, however, it was found expedient to chromatographically separate the photo-oxygenation products into two broad fractions containing monohydroperoxides **3a** and **4a**, and dihydroperoxides **5a** and **6a**. Thus the fractions containing hydroperoxides **5a** and **6a** on acid-catalyzed condensation with acetone followed by reduction with $NaBH_4$ in methanol furnished mixture of trioxanes **7a** and **8a** which were separated by column chromatography on silica gel. Trioxanes **7b–d** and **8b–d** were obtained by similar reaction of these hydroperoxides with cyclopentanone, cyclohexanone, and 2-adamantanone.[11] Trioxanes **8a–d** are formed from **6a** by acid-catalyzed cleavage of tertiary hydroperoxy group[5,6] ('Hock cleavage', Scheme 1). Trioxanes **7a–d**, though expected to be mixtures of diastereomers because of their origin from a diastereomeric

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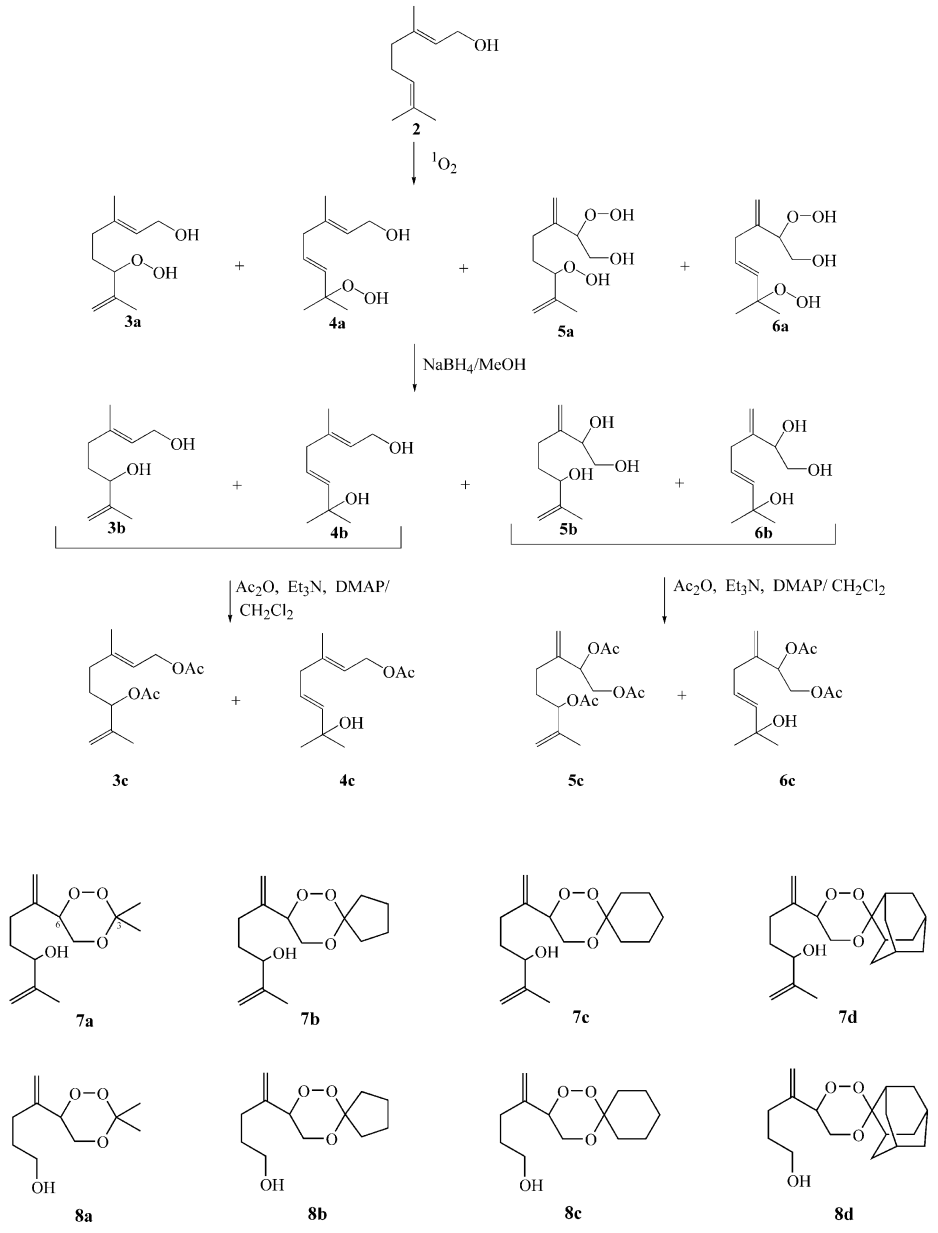


mixture of hydroperoxides **5a**, were found to be homogeneous by TLC, ^{1}H NMR and ^{13}C NMR.[11] It appears that they are mixtures of diastereomers with almost identical physicochemical properties.

Anti-Malarial Activity

The anti-malarial activity of trioxanes **7a**–**d** and **8a**–**d** was assessed against *Plasmodium falciparum* (NF-54 strain) in vitro cultures[7] using minor modification to the technique of Rieckmann and co-workers.[8] The results are summarized in Table 1.

As can be seen from Table 1, trioxanes having the adamantane moiety (**7d** and **8d**) are the most active while those with 3,3-dimethyl substituent (**7a** and **8a**) are least

Table 1. In vitro anti-malarial activity of trioxanes against *P. falciparum* (NF-54 strain)

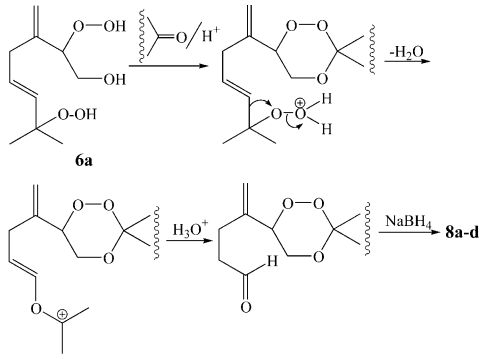
Compd	MIC[a] (ng/mL)
7a	500[b]
7b	125[b]
7c	125[b]
7d	31[b]
8a	1000[b]
8b	250[b]
8c	1000[b]
8d	125[b]
Artemisinin	31[b]
Chloroquine	40[c]

[a]MIC, minimum concentration inhibiting development of ring-stage parasites into the schizonts.
[b]Test was carried out at the following concentrations (2-fold serial dilution): 1000, 500, 250, 125, 62, 31, 15.5 ng/mL.
[c]Earlier determined standard value.

Table 2. In vivo anti-malarial response of trioxane **7d** against multi-drug resistant *P. yoelii* in Swiss mice

Dose (mg/kg/day)	Percent parasitemia (mean ± SE) on day[a]			No. of mice protected[b]
	4	7	10	
96	Nil (5)	Nil (5)	Nil (5)	5/5
48	Nil (5)	2.04 ± 0.9 (5)	10.5 ± 5.7 (4)	4/5
24	2.42 ± 0.8 (5)	13.38 ± 6.7 (5)	28.4 ± 17.4 (2)	2/5
Chloroquine 48	1.53 ± 0.4 (5)	17.6 ± 6.8 (5)	32.74 ± 11.2 (3)	3/5
Vehicle Control	7.50 ± 0.7 (5)	53.00 ± 6.9 (2)	Died	0/5

[a]Number of surviving animals are indicated in parentheses.
[b]Observation till day 10 post-infection.



Scheme 1. Mechanism of formation of trioxanes **8a**–**d**.

active. Also within a given substitution pattern at C-3 of trioxane, the trioxanes with the bulkier substituent at C-6 (**7a**–**d**) are more active than the ones having smaller substituents (**8a**–**d**), indicating that the anti-malarial activity could be improved by increasing the hydrophobicity, that is introducing bulkier group at C-6. **7d** is the most active compound of the series and its activity is comparable to that of artemisinin in vitro. Trioxane **7d** was also evaluated for its anti-malarial efficacy in vivo against multi-drug resistant *Plasmodium yoelii* in Swiss mice at 96, 48, and 24 mg/kg by in route[7] (Table 2).

As can be seen from Table 2, trioxane **7d** shows promising activity against multi-drug resistant *P. yoelii* in Swiss mice.

In conclusion, we have prepared a series of novel 1,2,4-trioxanes using an abundantly available natural product, which show promising anti-malarial activity. The novel feature of these trioxanes is the side chain with a hydroxyl group. This hydroxyl group offers further choice for making new derivatives of these trioxanes. Work on these lines is currently in progress in our laboratory.

Acknowledgement

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References and Notes

1. (a) For reviews on artemisinin and its derivatives, see: Lin, A. J.; Klayman, D. L.; Melthous, W. K. *J. Med. Chem.* **1987**, *30*, 2147. (b) Luo, X. D.; Shen, C. C. *Med. Res. Rev.* **1987**, *7*, 29. (c) Bross, A.; Venugopalan, B.; Gerpe, L. D.; Veh, H. J. C.; Anderson, J. L. F.; Buchs, P.; Luo, X. D.; Melhous, W.; Peters, W. *J. Med. Chem.* **1988**, *31*, 645. (d) Zaman, S. S.; Sharma, R. P. *Heterocycles* **1991**, *32*, 1593. (e) Barradell, L. B.; Fitton, A. *Drugs* **1995**, *50*, 714. (f) Li, Q.-G.; Peggins, J. O.; Lin, A. J.; Masonic, K. J.; Troutman, K. M.; Brewer, T. G. *Trans. Royal Soc. Trop. Med. Hyg.* **1998**, *92*, 332. (g) Bhattacharya, A. K.; Sharma, R. P. *Heterocycles* **1999**, *51*, 1681, and references cited therein.
2. (a) Synthesis of 1,2,4-trioxanes: Payne, G. B.; Smith, C. W. *J. Org. Chem.* **1957**, *22*, 1682. (b) Jefford, C. W.; Jaggi, D.; Boukouvalas, J.; Burger, S. *J. Am. Chem. Soc.* **1983**, *105*, 6497. (c) Singh, C. *Tetrahedron Lett.* **1990**, *33*, 6901. (d) Posner, G. H.; Oh, C. H.; Milhous, W. K. *Tetrahedron Lett.* **1991**, *34*, 4235. (e) Bloodworth, A. J.; Curtis, R. J.; Spencer, M. D.; Tallant, M. S. *Tetrahedron* **1993**, *49*, 2729.
3. (a) In vivo active anti-malarial trioxanes: Jefford, C. W.; Goran, M.; Ernest, C.; Boukouvalas, J.; Richardson, G.; Robinson, B. *Helv. Chim. Acta* **1988**, *71*, 1805. (b) Singh, C.; Mishra, D.; Saxena, G.; Chandra, S. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 497; **1995**, *5*, 1913. (c) Posner, G. H.; Jeon, H. B.; Parker, M. H.; Krasavin, M.; Paik, I.-H.; Shapiro, T. A. *J. Med. Chem.* **2001**, *44*, 3054.
4. Kinetics of photo-oxygenation of geraniol have been studied earlier, but the full characterization of the photo-oxygenation products have not been done: Tanielian, C.; Chaineaux, J. *Eur. Polym. J.* **1980**, *16*, 619.
5. Wistuba, E.; Ruchardt, C. *Tetrahedron Lett.* **1981**, *22*, 3389.
6. Yablokov, V. A. *Russ. Chem. Rev.* **1980**, *49*, 833.
7. **In vitro anti-malarial efficacy test:** The asynchronous parasites obtained from cultures of *P. falciparum* were synchronized after 5% sorbitol treatment so as to contain only ring-stage parasites.[9] Parasite suspension in medium RPMI 1640 at 1–2% parasitemia and 3% hematocrit was dispensed into wells of sterile 96-well plates. Test compounds were serially diluted in duplicate wells to obtain final test concentration. The culture plates were incubated in a candle jar at 37 °C for 36–40 h. Thin blood smears from each well prepared at the end of incubation period were microscopically examined and the concentration which inhibited the maturation of rings into schizonts stage was recorded as MIC.

In vivo anti-malarial efficacy test: The in vivo efficacy of compound **7d** was evaluated against *P. yoelii* (MDR) in Swiss mice model at 96, 48, and 24 mg/kg/day. The mice were inoculated with 1×10^6 parasitized RBC on day zero and treatment was administered to a group of five mice at each dose, from days 0 to 3, in two divided doses daily. The required drug dilutions were prepared in groundnut oil and

0.1 mL volume was administered intramuscularly for each dose. Parasitemia levels were recorded from thin blood smears between days 4 and 10.[10] All the mice of the control group (untreated) died by day 10.

8. Rieckmann, K. H.; Campbell, G. H.; Sax, L. J. U.; Mrema, J. E. *Lancet* **1978**, *1*, 22.
9. Lambros, C.; Vanderberg, J. P. *J. Parasitol.* **1979**, *65*, 418.
10. Puri, S. K.; Singh, N. *Expl. Parasit.* **2000**, *94*, 8.
11. Selected spectral data: Compound **5c**: FT-IR (neat, cm^{-1}) 1743. ^1H NMR (200 MHz, $CDCl_3$) δ 1.73 (s, 3H), 1.80–1.89 (m, 2H), 2.01–2.10 (m, 2H), 2.05 (s, 3H), 2.07 (s, 3H), 2.09 (s, 3H), 4.09 (dd, 1H, $J=11.8$, 7.6 Hz), 4.24 (dd, 1H, $J=11.8$, 3.4 Hz), 4.91 (s, 1H), 4.95 (s, 1H), 5.00 (s, 1H), 5.13 (s, 1H), 5.10–5.20 (m, 1H), 5.34–5.38 (m, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 17.71, 17.79, 20.41, 20.66, 20.80, 28.12, 28.23, 30.27, 64.07, 73.30, 73.41, 76.11, 76.37, 112.58, 112.77, 142.36, 142.52, 143.35, 169.60, 169.86, 170.28. MS (*m*/*z*) 312 (M^+), 252 (M^+-AcOH), 192 (252−AcOH), 132 (192−AcOH). Trioxane **7a**: ^1H NMR (200 MHz, $CDCl_3$) δ 1.38 (s, 3H), 1.64 (s, 3H), 1.66–1.69 (m, 2H), 1.73 (s, 3H), 2.06–2.16 (m, 2H), 3.75 (dd, 1H, $J=11.9$, 3.1 Hz), 3.94 (dd, 1H, $J=11.9$, 10.2 Hz), 4.07 (t, 1H, $J=6.2$ Hz), 4.69 (dd, 1H, $J=10.2$, 3.1 Hz), 4.86 (s, 1H), 4.95 (s, 1H), 5.06 (s, 2H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 17.99, 20.49, 25.76, 30.14, 33.48, 63.34, 75.53, 81.50, 102.68, 111.60, 114.37, 143.91, 147.62. MS (*m*/*z*) 242 (M^+), 210 (M^+-O_2), 152 (210−Me_2CO), 134 (152−H_2O), 111 (152−C_3H_5). Trioxane **7b**: ^1H NMR (200 MHz, $CDCl_3$) δ 1.68–1.97 (m, 9H), 1.73 (s, 3H), 2.05–2.19 (m, 2H), 2.40–2.51 (m, 1H), 3.82 (d, 2H, $J=6.0$ Hz), 4.06 (t, 1H, $J=6.1$ Hz), 4.76 (t, 1H, $J=6.0$ Hz), 4.85 (s, 1H), 4.95 (s, 1H), 5.04 (s, 2H). MS (*m*/*z*) 268 (M^+), 236 (M^+-O_2), 152 (236−C_5H_8O), 134 (152−H_2O), 111 (152−C_3H_5). Trioxane **7d**: ^1H NMR (200 MHz, $CDCl_3$) δ 1.55–2.15 (m, 17H), 1.73 (s, 3H), 2.90 (bs, 1H), 3.73 (dd, 1H, $J=11.8$, 3.1 Hz), 3.94 (dd, 1H, $J=11.8$, 10.3 Hz), 4.06 (t, 1H, $J=6.2$ Hz), 4.72 (dd, 1H, $J=10.3$, 3.1 Hz), 4.85 (s, 1H), 4.95 (s, 1H), 5.05 (s, 2H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 18.00, 27.54 (two CH), 29.76, 30.24, 33.37, 33.51, 33.63, 33.85, 33.93, 36.54, 37.59, 62.10, 75.46, 81.46, 104.87, 111.55, 114.42, 144.11, 147.65. MS (*m*/*z*) 334 (M^+), 302 (M^+-O_2), 152 (302−$C_{10}H_{14}O$), 134 (152−H_2O), 111 (152−C_3H_5). Trioxane **8a**: ^1H NMR (200 MHz, $CDCl_3$) δ 1.38 (s, 3H), 1.64 (s, 3H), 1.70–1.80 (m, 2H), 2.16 (t, 2H, $J=7.5$ Hz), 3.67 (t, 1H, $J=6.3$ Hz), 3.75 (dd, 1H, $J=11.9$, 3.0 Hz), 3.95 (dd, 1H, $J=11.9$, 10.1 Hz), 4.40 (dd, 1H, $J=10.1$, 3.0 Hz), 5.19 (s, 2H). MS (*m*/*z*) 202 (M^+), 170 (M^+-O_2), 112 (170−Me_2CO), 94 (112−H_2O). Trioxane **8c**: ^1H NMR (200 MHz, $CDCl_3$) δ 1.55–2.21 (m, 14H), 3.67 (t, 2H, $J=6.3$ Hz), 3.73 (dd, 1H, $J=11.6$, 3.0 Hz), 3.97 (dd, 1H, $J=11.6$, 10.5 Hz), 4.76 (dd, 1H, $J=10.5$, 3.0 Hz), 5.06 (s, 2H). MS (*m*/*z*) 242 (M^+), 210 (M^+-O_2), 112 (210−$C_6H_{10}O$), 94 (112−H_2O). Trioxane **8d**: ^1H NMR (200 MHz, $CDCl_3$) δ 1.55–2.30 (m, 17H), 2.90 (bs, 1H), 3.67 (t, 2H, 6.3 Hz), 3.73 (dd, 1H, $J=11.7$, 3.0 Hz), 3.95 (dd, 1H, $J=11.7$, 10.5 Hz), 4.72 (dd, 1H, $J=10.5$, 3.0 Hz), 5.06 (s, 2H). MS (*m*/*z*) 294 (M^+), 262 (M^+-O_2), 112 (262−$C_{10}H_{14}O$), 94 (112−H_2O).