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Chemistry of 1,2,4-trioxanes relevant to their mechanism of action. Part 1: Reaction with Fe(II) salts☆

Chandan Singh,* Nitin Gupta and Pallavi Tiwari

Division of Medicinal and Process Chemistry, Central Drug Research Institute, Lucknow 226 001, India

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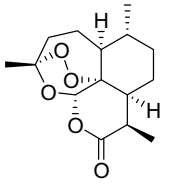
Abstract—Reactions of 6-(1′-phenylvinyl)-1,2,4-trioxanes **2**–**5**, with $FeCl_2{\cdot}4H_2O$, $FeBr_2$, and a combination of hemin (bovine) and reduced glutathione (GSH) under various conditions have been studied.

With its unusual and unique structure, high antimalarial efficacy and negligible toxicity artemisinin **1** has fascinated both chemists and biologists for the past three decades.[1] Artemisinin and its derivatives are the only class of antimalarials against which no clinically relevant resistance has been reported. Several studies conducted on **1** have clearly proved the essential requirement for a peroxide linkage for the antimalarial activity. However, due to several disadvantages associated with **1** such as limited availability from natural sources, high cost, and poor oral bioavailability, there have been concerted efforts from several research groups to prepare structurally simpler 1,2,4-trioxanes and other peroxides as substitutes for artemisinin and its derivatives.[2]

Artemisinin is active at the blood stage of the malarial infection. Since parasite-invaded erythrocytes are rich in heme, it is believed that a possible mode of action of artemisinin involves its interaction with the heme Fe(II). Several studies involving the Fe(II)-mediated chemistry of artemisinin and synthetic 1,2,4-trioxanes which support this hypothesis have been reported.[1e,2a,3]



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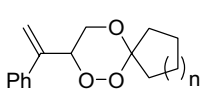
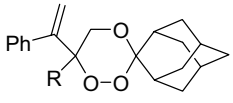
Aiming to develop structurally simple substitutes for artemisinin, we earlier reported a convenient and high yielding procedure for the synthesis of 1,2,4-trioxanes.[4] A unique feature of these trioxanes is the presence of a 1-substituted vinyl group at C-6 of the trioxane. Several of these 1,2,4-trioxanes have shown promising antimalarial activity both in vitro and in vivo.[4,5] As part of our efforts to understand the mechanism of action of these trioxanes we have now studied the reactions of trioxanes **2**–**5** with $FeCl_2{\cdot}4H_2O$, $FeBr_2$, and a combination of hemin (bovine) and GSH under various conditions. Herein, we report the results of this study and the relevance of these results to the mechanism of action of these trioxanes.



2, n=1
3, n=2



4, R=H
5, R=Me

The reaction of trioxane **2** with $FeBr_2$ (0.5 equiv) in anhydrous THF was complete in 15 min and gave bromoester **6** (30% yield) as the only isolable product (entry 1, Table 1). Addition of water (6 equiv) or L-(+)-ascorbic acid (1.2 equiv) did not affect product composition and gave **6** in 47% and 41% yields, respectively (entries 2 and 3, Table 1).[6] The reaction of **2** with $FeCl_2{\cdot}4H_2O$ (1.0 equiv) in MeCN was comparably much slower (24 h for completion) and gave chloroester **7** (19% yield) as the only isolable product (entry 4, Table 1). Trioxane **3** behaved similarly both with $FeBr_2$ and $FeCl_2{\cdot}4H_2O$. Thus reaction of **3** with $FeBr_2$ (0.5 equiv)

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* Corresponding author. Tel.: +91 522 2624273; fax: +91 522 2623405; e-mail: chandancdri@yahoo.com

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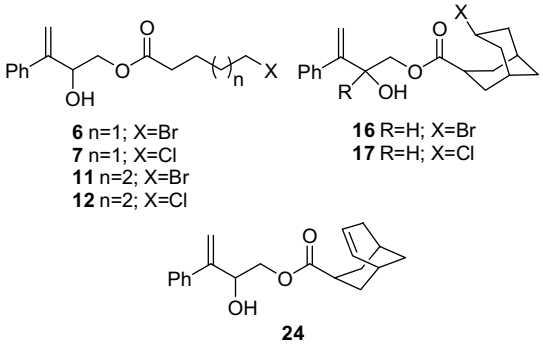
Table 1. FeX_2-mediated reactions of trioxanes **2**–**4** at rt

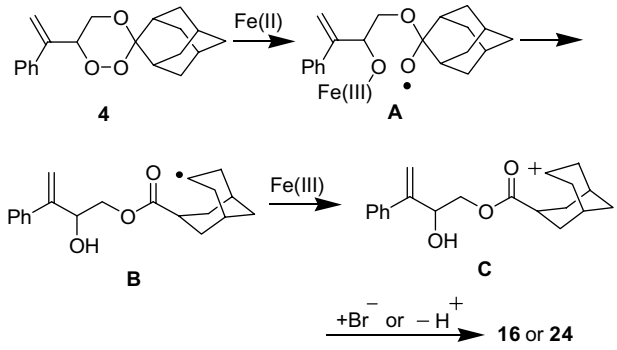
Entry	Trioxane	Fe(II) reagent (equiv)	Additive (equiv)	Solvent	Time (h)	Products (% yield)
1	**2**	$FeBr_2$ (0.5)	—	THF	0.25	**6** (30)
2	**2**	$FeBr_2$ (0.5)	H_2O (6)	THF	0.25	**6** (47)
3	**2**	$FeBr_2$ (0.5)	L-(+)-Ascorbic acid (1.2)	THF	0.25	**6** (41)
4	**2**	$FeCl_2{\cdot}4H_2O$ (1.0)	—	MeCN	24	**7** (19)
5	**3**	$FeBr_2$ (0.5)	—	THF	0.25	**11** (20)
6	**3**	$FeBr_2$ (0.5)	H_2O (6)	THF	0.25	**11** (31)
7	**3**	$FeCl_2{\cdot}4H_2O$ (1.0)	—	MeCN	24	**12** (7)
8	**4**	$FeBr_2$ (0.5)	—	THF	0.25	**16** (33), **24** (18)
9	**4**	$FeBr_2$ (0.5)	H_2O (6)	THF	0.25	**16** (48), **24** (10)
10	**4**	$FeCl_2{\cdot}4H_2O$ (1.0)	—	MeCN	8	**17** (4), **24** (2)

in THF was complete within 15 min and furnished bromoester **11**, as the only isolable product in 20% yield. Addition of water (6 equiv) to the reaction mixture improved the yield and gave **11** in 31% yield. Reaction of **3** with $FeCl_2{\cdot}4H_2O$ was also slow and furnished chloroester **12** in only 7% yield (Table 1).

The reaction of adamantane-based trioxane **4** with $FeBr_2$ (0.5 equiv) in THF furnished bromoester **16** as an inseparable 1:1 mixture of diastereomers in 33% yield and olefinester **24** also as an inseparable 1:1 mixture of diastereomers in 18% yield. Addition of water (6 equiv) to the reaction mixture improved the yield of **16** to 48% while olefinester **24** was isolated in 10% yield. The reaction of trioxane **4** with $FeCl_2{\cdot}4H_2O$ was extremely slow and after 8 h chloroester **17** and olefinester **24** could be isolated in only 4% and 2% yields, respectively (Table 1).



Formation of **6** and **7** from trioxane **2**, **11** and **12** from trioxane **3**, and **16**, **17**, and **24** from **4** are consistent with initial reduction of the peroxide group with Fe(II) to an oxyradical anion **A** as exemplified for trioxane **4** in Scheme 1. The oxyradical anion **A** would pick up a proton and rearrange to the C-centered radical **B** which on subsequent oxidation with Fe(III) would lead to carbocation **C**, which is then trapped with a nucleophile (Br^- or Cl^-) to furnish **16** or **17**, while loss of a proton from **C** would give olefin **24** (Scheme 1). This scheme is consistent with a similar reaction of $FeCl_2{\cdot}4H_2O$ with synthetic trioxanes as reported by Jefford.[2a] The C-centered radical **B** and the carbocation **C** can also react with the styrene group, present in both the reactant and the products, leading to polymeric side products and this could be the reason for the poor mass balance observed in most of the reactions.



Scheme 1. Mechanism of FeX_2-mediated reactions.

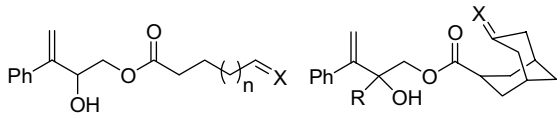
Having studied the reactions of the trioxanes **2**–**4** with $FeBr_2$ and $FeCl_2{\cdot}4H_2O$ we also examined the reaction of these trioxanes with a hemin–GSH combination, conditions which are closer to the in vivo system. While the reactions of artemisinin with hemin have been studied earlier,[3] similar studies with synthetic trioxanes have not been reported.

The reaction of trioxane **2** with a catalytic amount (0.1 equiv) of hemin and GSH (2 equiv) in MeCN–H_2O (3:2) under aerobic conditions was sluggish and was not complete even after 24 h at rt. Chromatography of the reaction mixture furnished ester **8** (3%), diol **9** (2%), aldehyde **10** (14%), and unreacted trioxane **2** (32%) (Table 2). Trioxane **3** on reaction under similar conditions furnished ester **13** (5%), diol **14** (4%), aldehyde **15** (14%), and trioxane **3** (20%). Reaction of the adamantane-based trioxane **4** with hemin/GSH/air in THF–H_2O (3:2) furnished diol **19** (24%), ketone **20** (2%), and unreacted trioxane **4** (40%). Reaction of another adamantane-based trioxane **5** (with an additional C-6 methyl) with hemin/GSH/air in THF–H_2O (3:2) was extremely sluggish and gave diol **22** and keto alcohol **23** in 10% and 2% yields, respectively; 60% of the trioxane **5** was recovered unchanged (Table 2).[6]

The results of reactions of trioxanes **2**–**5** with hemin/GSH were qualitatively different when the reactions were conducted under an argon atmosphere as none of the oxygenated products **9**, **10**, **14**, **15**, **19**, **20**, **22**, and **23** were detected in the reaction mixture. Instead, trioxanes **2**, **3**, **4**, and **5** gave **8** (9%), **13** (10%), **18** (8%), and **21** (8%), respectively (Table 2).

Table 2. Hemin/GSH-mediated reactions of trioxanes **2**–**5** at rt

Entry	Trioxane	Hemin (equiv)	GSH (equiv)	Atmosphere	Solvent	Time (h)	Products (% yield)
1	**2**	0.1	1.5	Air	MeCN–H_2O (3:2)	24	**8** (3), **9** (1.5), **10** (14), **2** (32)
2	**2**	0.1	1.5	Argon	MeCN–H_2O (3:2)	24	**8** (9)
3	**3**	0.1	1.5	Air	MeCN–H_2O (3:2)	24	**13** (5), **14** (4), **15** (14), **3** (20)
4	**3**	0.1	1.5	Argon	MeCN–H_2O (3:2)	24	**13** (10)
5	**4**	0.1	1.5	Air	THF–H_2O (3:2)	24	**19** (24), **20** (2), **4** (40)
6	**4**	0.1	1.5	Argon	THF–H_2O (3:2)	24	**18** (8), **4** (16)
7	**5**	0.1	1.5	Air	THF–H_2O (3:2)	48	**22** (10), **23** (2), **5** (60)
8	**5**	0.1	1.5	Argon	THF–H_2O (3:2)	24	**21** (8), **5** (34)



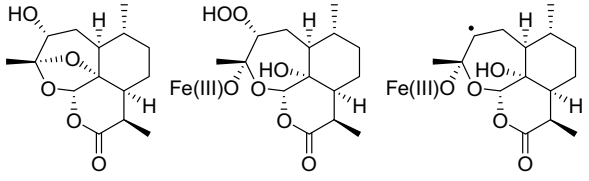
8 n=1; X=H,H
9 n=1; X=H,OH
10 n=1; X=O
13 n=2; X=H,H
14 n=2; X=H,OH
15 n=2; X=O

18 R=H; X=H,H
19 R=H; X=H,OH
20 R=H; X=O
21 R=Me; X=H,H
22 R=Me; X=H,OH
23 R=Me; X=O

Clearly, molecular oxygen is participating in the formation of oxygenated products. Thus the carbon-centered free radical **B** formed on fragmentation of the C–C bond of **A** is being trapped by molecular oxygen to furnish hydroperoxide **D** which on further reaction with Fe(II) gives **19** and **20** (Scheme 2). Such observation have not been reported earlier with synthetic trioxanes and the formation of these products or the intermediate hydroperoxides in the mechanism of action of these trioxanes remains to be explained.

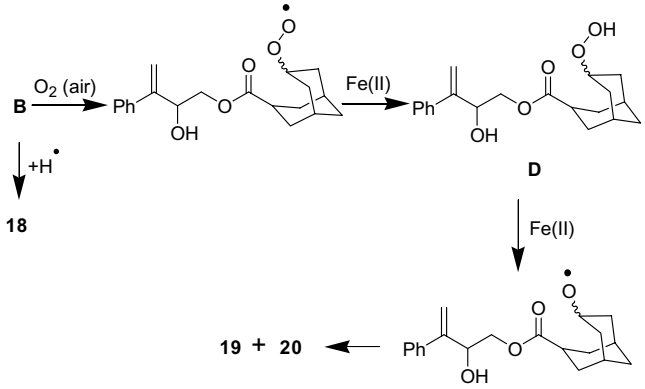
The intermediate hydroperoxide itself could be the biologically active species as it could put the malaria parasite under oxidative stress. Out of the four trioxanes (**2**–**5**) studied here trioxanes **2**–**4** have shown significant antimalarial activity in vivo against *Plasmodium berghei* in mice while trioxane **5** has been found to be inactive in the same model.[5a,b] Trioxanes **4** and **5**, which are structurally very close, give similar products on reaction with hemin/GSH except that the inactive trioxane **5** reacted at a much slower rate. Clearly, data on more compounds is needed to correlate the antimalarial activity with the products of the reaction of the trioxanes with hemin/GSH.

In this context it is worth mentioning that the origin of the major product **25** of the reaction of artemisinin **1** with the Fe(II) salts[3e] could well be the hydroperoxide **26** formed by the entrapment of secondary radical **27** with molecular oxygen.



25 **26** **27**

In conclusion, in our efforts to elucidate the mechanism of 6-(1′-arylvinyl)-1,2,4-trioxanes we have studied the reactions of trioxanes **2**–**5** with $FeCl_2{\cdot}4H_2O$, $FeBr_2$, and hemin/GSH. While the results of the reaction of these trioxanes with $FeCl_2{\cdot}4H_2O$, and $FeBr_2$ are similar to that observed by other workers, the products formed with hemin/GSH, especially under aerobic conditions, are new and interesting as the formation of these products requires the intermediacy of hydroperoxides which themselves could be potentially toxic to the parasite.



Scheme 2. Mechanism of hemin/GSH-mediated reactions.

Acknowledgements

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6. Selected spectral data. Compound **6**: oil; IR (neat, cm^{-1}) 1630, 1729, 3460; ^1H NMR (300 MHz, $CDCl_3$) δ 1.78 (m, 2H), 1.89 (m, 2H), 2.36 (t, 2H, $J = 7.5$ Hz), 2.47 (bs, 1H, OH), 3.40 (t, 2H, $J = 6.3$ Hz), 4.03 (dd, 1H, $J = 11.7$, 7.5 Hz), 4.21 (dd, 1H, $J = 11.7$, 2.7 Hz), 4.91 (bm, 1H), 5.45 (s, 1H), 5.50 (s, 1H), 7.34 (m, 5H); FAB-MS (*m*/*z*) 327 and 329 $[M+H]^+$. Compound **15**: oil; IR (neat, cm^{-1}) 1726, 3448; ^1H NMR (300 MHz, $CDCl_3$) δ 1.59–1.66 (m, 4H), 2.35 (t, 2H, $J = 7.0$ Hz), 2.47 (t, 2H, $J = 7.0$ Hz), 2.60 (bs, 1H, OH), 4.02 (dd, 1H, $J = 11.7$, 7.8 Hz), 4.20 (dd, 1H, $J = 11.7$, 3.0 Hz), 4.92 (bm, 1H), 5.45 (s, 1H), 5.51 (s, 1H), 7.27–7.37 (m, 5H), 9.77 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 21.78 (t), 24.67 (t), 34.19 (t), 43.82 (t), 68.30 (t), 72.16 (d), 114.85 (t), 127.04 (2 × d), 128.38 (d), 128.94 (2 × d), 139.53 (s), 148.01 (s), 173.85 (s), 202.45 (d); FAB-MS (*m*/*z*) 277 $[M+H]^+$. Compound **16**: oil; IR (neat, cm^{-1}) 1633, 1721, 3440; ^1H NMR (300 MHz, $CDCl_3$) δ 1.25 (bm, 1H), 1.42–1.63 (m, 2H), 1.68–1.91 (m, 2H), 1.99–2.71 (m, 9H), 4.02 (dd, 1H, $J = 11.7$, 7.5 Hz), 4.21 and 4.33 (2 × dd, 1H, $J = 11.7$, 2.7 Hz each), 4.35–4.50 (m, 1H), 4.91 and 5.00 (2 × bm, 1H), 5.45 and 5.46 (2 × s, 1H), 5.50 and 5.53 (2 × s, 1H), 7.29–7.43 (m, 5H); FAB-MS (*m*/*z*) 393 and 395 $[M+H]^+$. Compound **19**: oil; IR (neat, cm^{-1}) 1632, 1722, 3399; ^1H NMR (200 MHz, $CDCl_3$) δ 1.10–1.35 (m, 3H), 1.46–1.59 (m, 4H), 1.89 (bs, 1H, OH), 1.95 (bs, 1H, OH), 2.03–2.19 (m, 4H), 2.52 (m, 2H), 3.99 (m, 2H), 4.19 (dd, 1H, $J = 11.6$, 3.0 Hz), 4.90 (bm, 1H), 5.44 (s, 1H), 5.49 (s, 1H), 7.34 (m, 5H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 26.25 (2 × d), 29.13 (t), 29.79 (2 × t), 35.98 (d), 42.79 (2 × t), 64.45 (d), 68.15 (t), 72.23 (d), 114.84 (t), 127.06 (2 × d), 128.37 (d), 128.94 (2 × d), 139.57 (s), 148.12 (s), 177.27 (s); FAB-MS (*m*/*z*) 331 $[M+H]^+$. Compound **23**: oil; IR (neat, cm^{-1}) 1723, 3428; ^1H NMR (300 MHz, $CDCl_3$) δ 1.40 (s, 3H), 1.50–1.90 (m, 4H), 2.24–2.68 (m, 9H), 3.68 (bs, 1H, OH), 4.04 (d, 1H, $J = 11.7$ Hz), 4.14 (d, 1H, $J = 11.7$ Hz), 5.10 (d, 1H, $J = 1.5$ Hz), 5.64 (d, 1H,$J = 1.5$ Hz), 7.30 (m, 5H); FAB-MS (*m*/*z*) 343 $[M+H]^+$. Compound **24**: oil; IR (neat, cm^{-1}) 1636, 1710, 3411; ^1H NMR (300 MHz, $CDCl_3$) δ 1.53 (bm, 1H), 1.65–1.77 (m, 3H), 2.06–2.58 (m, 7H), 2.77 (bs, 1H, OH), 3.89 and 3.94 (2 × dd, 1H, $J = 11.7$, 7.8 Hz each), 4.10 and 4.16 (2 × dd, 1H, $J = 11.7$, 2.7 Hz each), 4.90 (bm, 1H), 5.45 (s, 1H), 5.52 (s, 1H), 5.55 (bs, 1H), 5.60 (bm, 1H), 7.29–7.41 (m, 5H); FAB-MS (*m*/*z*) 313 $[M+H]^+$.