Differentiation between 1,2,4,5-Tetraoxanes and 1,2,4,5,7,8-Hexaoxonanes Using ^{1}H and ^{13}C NMR Analyses

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The proton and carbon resonance spectra of ten pairs of 1,2,4,5-tetraoxanes and 1,2,4,5,7,8-hexaoxonanes derived from acyclic and cyclic ketones have been recorded. A reliable method for distinguishing between 1,2,4,5-tetraoxanes and 1,2,4,5,7,8-hexaoxonanes by inspection of their chemical shifts, signal number, signal shape, and dynamic features has been developed. This method provides a rapid structural assignment of 1,2,4,5-tetraoxanes and 1,2,4,5,7,8-hexaoxonanes without using vapor pressure osmometry, isotope labeling or low temperature NMR techniques.

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1,2,4,5-Tetraoxanes and 1,2,4,5,7,8-hexaoxonanes can be synthesized by acid-catalyzed peroxidation of ketones [1]. In some cases, the procedure developed for the preparation of tetraoxanes produces instead a mixture of tetraoxanes and hexaoxonanes [2] or only hexaoxonanes [3]. Therefore, it is important to distinguish between these oligomers. A reliable and commonly adopted method is vapor pressure osmometry (VPO) molecular weight analysis, as the molecular weights of tetraoxanes and hexaoxonanes differ by a third. Obviously, the VPO method cannot determine ratios of a mixture of those oligomers accurately, nor is it suitable for the analysis of crude reaction products. Story *et al.* [4] examined the thermal decomposition of over one hundred tetraoxanes and hexaoxonanes, and could in some cases verify their structures and assess the tetraoxane/hexaoxonane ratio based on the identification of macrocyclic hydrocarbon and lactone decomposition products. The practical use of this thermolysis method for structural analysis is limited because of the low to moderate yields of the decomposition products.

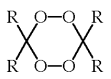
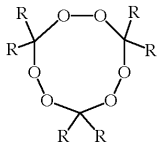
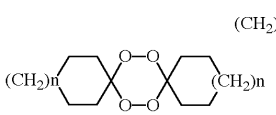
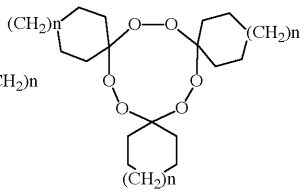
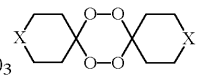
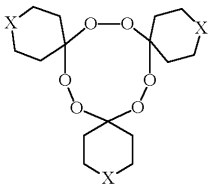
Unexpectedly, spectroscopic methods for distinguishing between tetraoxanes and hexaoxonanes have not been successfully employed. IR spectroscopy has proved to be insufficiently specific [4]. Mass spectroscopy has been considered as a tool to differentiate between these two classes of peroxides, but unfortunately, molecular ions are not always present, and are extremely weak when detected, due possibly to rapid fragmentation [5]. Although ^{1}H NMR spectroscopy has been widely used to characterize tetraoxanes, [3] published ^{13}C NMR data of tetraoxanes and ^{1}H and ^{13}C NMR data of hexaoxonanes are rare, and a systematic comparison of ^{1}H and ^{13}C NMR spectra of these oligomeric peroxides has not been reported. As part of our work studying the chemistry and biology of peroxide antimalarial agents, [6] we have been interested in the synthesis of tetraoxanes and hexaoxonanes. In this paper, we describe a simple and rapid method to differentiate tetraoxanes and hexaoxonanes using classic ^{1}H and ^{13}C NMR techniques.

Results and Discussion.

Synthesis of Tetraoxanes and Hexaoxonanes.

We set out to prepare ten pairs of structurally diverse tetraoxanes and hexaoxonanes (Table 1) that would provide sufficient NMR data. All of the peroxides, fifteen of which are known compounds, were synthesized *via* literature procedures (*vide* experimental section), and were characterized by ^{1}H and ^{13}C NMR spectra, and elemental and VPO molecular weight analyses.

Table 1
Structures of Tetraoxanes (**1t-10t**) and Hexaoxonanes (**1h-10h**)

Pairs	R, n or X	Tetraoxanes (t)	Hexaoxonanes (h)
1	R = Me		
2	Pr		
3	Bu		
4	Pentyl		
5	n = 0		
6	1		
7	2		
8	X = O		
9	CHMe		
10	$CH(Me)_3$		

Structural Assignment by ^{1}H NMR.

We made an assumption that all tetraoxanes adopt a chair conformation and all hexaoxonanes a D3 conformation based on X-ray determinations of several tetraoxanes and hexaoxonanes [7]. This conformational difference

should in principle make for distinct spectra. It is known that in the chair conformation, equatorial and axial substituents are deshielded and shielded, respectively, [8] and in the D3 conformation, substituents are magnetically identical [9]. Due to overlapping axial substituent signals with the remaining alkyl group envelope signals, we focused only on signals of equatorial substituents in tetraoxanes, and signals of substituents adjacent to the hexaoxonane ring in hexaoxonanes. In each case, these diagnostic signals (Table 2) were the most downfield peaks in the proton NMR spectra.

Table 2
Proton Chemical Shifts of Diagnostic Signals and their Differences

R, n or X	Compound	δ	Δδ
R = Me	**1t**	1.80 (br s, 6H)	0.34
	1h	1.46 (s, 18H)	
R = Pr	**2t**	2.17 (br s, 4H)	0.42
	2h	1.75 (m, 6H)	
R = Bu	**3t**	2.19 (br s, 4H)	0.42
	3h	1.77 (m, 6H)	
R = Pentyl	**4t**	2.20 (br s, 4H)	0.43
	4h	1.77 (m, 6H)	
n = 0	**5t**	2.42 (t, 4H)	0.18
	5h	2.24 (m, 6H)	
n = 1	**6t**	2.29 (br s, 4H)	0.47
	6h	1.82 (m, 6H+6H)	
n = 2	**7t**	2.42 (br s, 4H)	0.24
	7h	2.18 (m, 6H)	
X = O	**8t**	2.48 (br s, 4H)	0.47
	8h	2.01 (m, 6H)	
X = CHMe	**9t**	3.05 (br s, 2H)	0.80
	9h	2.21 (m, 6H)	
X = $CHC(Me)_3$	**10t**	3.19 (br s, 2H)	0.89
	10h	2.30 (m, 6H)	

In all cases, diagnostic tetraoxane signals appeared at higher frequencies than diagnostic hexaoxonane signals (column δ), and in comparison, were deshielded by 0.18 to 0.89 ppm (column Δδ). For **1**–**4**, this deshielding effect was nearly substituent-independent, whereas for **5**–**7**, the deshielding effect was a function of the size of the spiro ring. Notably, methyl and *t*-butyl groups on spiro rings in **9**–**10** caused a dramatic deshielding of 0.8-0.9 ppm. We attribute this substantial downfield shift to double deshielding effects, the one from the tetraoxane ring and the other from a substituted cyclohexane ring. That is, the inversion of the 4-substituted cyclohexane ring is slow in these two cases. It is worth noting that while the integration of diagnostic signals of tetraoxane **2t**–**8t** corresponds to 4 protons of two equatorial methylene groups, only 2 protons were represented by diagnostic signals of tetraoxane **9t**–**10t**, supporting the presence of a second deshielding (split). For hexaoxonanes **2h**–**10h**, the diagnostic signals consisted of half of an AB-system, corresponding to six protons of the diastereotopic [9] methylene groups adjacent to the spiro carbon atoms.

Structural Assignment by ^{13}C NMR.

We then examined tetraoxane and hexaoxonane ^{13}C NMR spectra to see how they differed from each other. We expected that the total signal number for tetraoxanes and hexaoxonanes would differ since the spiro equatorial and axial carbons in tetraoxanes are not magnetically equivalent, and the corresponding carbons adjacent to the triperoxide heterocycle in hexaoxonanes are magnetically identical. We also anticipated that some broad signals would appear for tetraoxanes due to conformational inversion, [10] but we expected only sharp signals for the relatively inflexible hexaoxanones [9]. Indeed, all spectra of tetraoxanes contained 2 or 4 broadened signals, whereas only sharp signals are observed for hexaoxonanes (Table 3). With exception of **9t** and **10t**, tetraoxanes **1t**-**8t** displayed more signals than their corresponding hexaoxonanes. The 2-fold increase in signal number for **9h** and **10h** could be interpreted by a syn/syn/anti orientation of methyl or *t*-butyl substituents in one stereoisomer or by the presence of two or more diastereomers.

Table 3
Total and Broad Carbon Signal Numbers

R, n or X	Compound	Total No	Br. No
R = Me	**1t**	3	2
	1h	2	0
R = Pr	**2t**	6	4
	2h	4	0
R = Bu	**3t**	7	4
	3h	5	0
R = Pentyl	**4t**	8	4
	4h	6	0
n = 0	**5t**	5	4
	5h	3	0
n = 1	**6t**	5	4
	6h	4	0
n = 2	**7t**	6	4
	7h	4	0
X = O	**8t**	5	4
	8h	3	0
X = CHMe	**9t**	9	4
	9h	18	0
X = $CHC(Me)_3$	**10t**	10	4
	10h	18	0

Structural Assignment by Dynamic Features.

Ten pairs of tetraoxanes and hexaoxonanes were investigated by using ^1H NMR at elevated temperatures. All tetraoxanes displayed significant changes in peak shape and chemical shifts between 20 and 60 °C; in the same temperature range, ^1H NMR spectra of hexaoxonanes were practically unaffected. Figure 1 illustrates the spectral changes as a function of temperature for **4t**. One can see the broadened signals for CH_2ax (1.59 ppm) and CH_2eq (2.20 ppm) at 20 °C. At 40 °C, coalescence occurs. At higher temperatures, inversion of the tetraoxane ring is fast on the time scale of the NMR experiments, and therefore both CH_2 protons give only one signal. The coalescence temperatures for other tetraoxanes lie between 40 and 60 °C, temperatures that can be

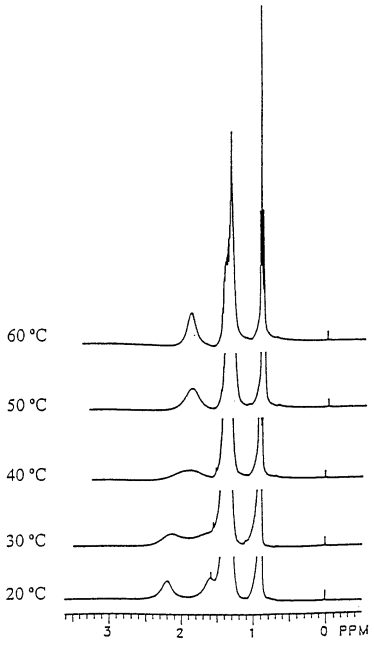


Figure 1. The 300 MHz ^{1}H NMR spectrum of **4t** as a function of temperature.

conveniently and rapidly accessed. The temperature-independent spectra of **1h**-**10h** indicate that the hexaoxonane ring is either rigid or highly flexible. In fact, the enantiomerization of an all-*cis* hexaoxonane derived from chloroacetone was reported to have a free-energy barrier of more than 24 kcal/mol, although the resolution of such molecules has not been accomplished.[9]

In conclusion, 1D NMR spectroscopic analysis is suitable for structural differentiation between tetraoxanes and hexaoxonanes based on chemical shifts, signal number, signal shape, and dynamic features. This method provides a rapid and reliable structural assignment without using vapor pressure osmometry, isotope labeling, or low temperature NMR techniques.

EXPERIMENTAL

The melting points are uncorrected. ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectra were recorded on a Varian XL-300 spectrometer using deuteriochloroform as a solvent. All chemical shifts are reported in parts per million (ppm) and are relative to internal tetramethylsilane (TMS) for ^{1}H and deuteriochloroform (77.0 ppm) for ^{13}C NMR. Microanalyses were performed by M-H-W-laboratories, Phoenix, AZ. Molecular weights were determined *via* the vapor-pressure osmometry (VPO) method by Galbraith Laboratories, Inc., Knoxville, TN and by Huffman Laboratories, Inc., Golden, CO. All ketones were purchased from Aldrich Chemical Co. Although we have encountered no difficulties in working with these relatively stable tetraoxanes and hexaoxonanes, [1b,1c] routine precautions such as the use of shields, fume hoods, and avoidance of metal salts should be observed whenever possible.

Synthesis of Tetraoxanes.

Tetraoxanes **1t**, **2t**, **3t**, **6t** and **7t** were prepared by a standard peroxidation method [3,11]. Tetraoxanes **4t**, **8t**, **9t** and **10t** were synthesized *via* ozonolysis of ketone *O*-methyl oximes [11]. Tetraoxane **5t** was synthesized according to the procedure of Sanderson *et al.* [12] using 70% H_2O_2. NMR data for **8t**, **9t** and **10t** were recently reported [11].

3,3,6,6-Tetramethyl-1,2,4,5-tetraoxane (**1t**).

Compound **1t** was obtained in 57% yield as a colorless solid; mp 133-134 °C (acetonotrile) (lit. [3] mp 133–135 °C); ^{1}H NMR: δ 1.36 (br s, 6H), 1.80 (br s, 6H); ^{13}C NMR: δ 20.48 (br s), 22.35 (br s), 107.48.

3,3,6,6-Tetrapropyl-1,2,4,5-tetraoxane (**2t**).

Compound **2t** was obtained in 56% yield as a colorless solid; mp 52–54 °C (acetonitrile) (lit. [13] mp 52–54 °C); ^{1}H NMR: δ 0.95 (br s, 12H), 1.20–1.51 (m, 8H), 1.52–1.70 (m, 4H), 2.17 (br s, 4H); ^{13}C NMR: δ 14.32, 15.46 (br s), 16.99 (br s), 33.25 (br s), 35.89 (br s), 110.45.

3,3,6,6-Tetrabutyl-1,2,4,5-tetraoxane (**3t**).

Compound **3t** was obtained in 41% yield as a colorless solid; mp 37–39 °C (acetonitrile); ^{1}H NMR: δ 0.92 (br s, 12H), 1.36 (br s, 16H), 1.50–1.75 (m, 4H), 2.19 (br s, 4H); ^{13}C NMR: δ 13.89, 22.89, 24.04 (br s), 25.80 (br s), 30.73 (br s), 33.42 (br s), 110.62. VPO MW (deuteriochloroform) 306; calcd MW 316.

Anal. Calcd. for $C_{18}H_{36}O_4$: C, 68.31; H, 11.47. Found: C, 68.54; H, 11.48.

3,3,6,6-Tetrapentyl-1,2,4,5-tetraoxane (**4t**).

Compound **4t** was obtained in 14% yield as a colorless solid; mp 33–35 °C (acetonitrile); ^{1}H NMR δ 0.91 (t, J = 7.1 Hz, 12H), 1.15–1.60 (m, 24H), 1.59 (br s, 4H), 2.20 (br s, 4H); ^{13}C NMR δ 13.93, 21.62 (br s), 22.45, 23.29 (br s), 30.94 (br s), 31.94, 33.80 (br s), 110.58. VPO MW (Chloroform) 349; calcd MW 373.

Anal. Calcd. for $C_{22}H_{44}O_4$: C, 70.92; H, 11.90. Found: C, 70.76; H, 11.70.

6,7,13,14-Tetraoxadispiro[4.2.4.2]tetradecane (**5t**).

Compound **5t** was obtained in 23% yield; mp 100–101, °C (acetonitrile) (lit. [14] mp 105 °C); ^{1}H NMR: δ 1.50–1.89 (m, 12H), 2.42 (t, J = 7.0 Hz, 4H); ^{13}C NMR: δ 23.90 (br s), 25.09 (br s), 34.34 (br s), 35.27 (br s), 119.72.

7,8,15,16-Tetraoxadispiro[5.2.5.2]hexadecane (**6t**).

Compound **6t** was obtained in 66% yield; mp 130–131 °C (acetonitrile) (lit. [3] mp 131–132 °C); ^{1}H NMR: δ 1.35–1.51 (m, 4H), 1.52–1.72 (m, 12H), 2.29 (br s, 4H); ^{13}C NMR: δ 21.94 (br s), 22.10 (br s), 25.38, 29.55 (br s), 31.77 (br s), 108.12.

8,9,17,18-Tetraoxadispiro[6.2.6.2]octadecane (**7t**).

Compound **7t** was obtained in 37% yield; colorless solid, mp 98–100 °C (acetonitrile); (lit. [15] mp 103 °C) ^{1}H NMR: δ 1.45–1.85 (m, 20H), 2.42 (br s, 4H); ^{13}C NMR: δ 22.48, 29.53 (br s), 30.19 (br s), 31.01 (br s), 35.97 (br s), 112.39.

Synthesis of Hexaoxonanes.

Hexaoxonane **1h** was produced by a neat peroxidation of acetone [1b]. Peroxidation of dipropyl ketone following the Cafferata procedure [2] afforded dihydroperoxide **11** which was

converted into hexaoxonane **2h** on treatment with dipropyl ketone and sulfuric acid. Hexaoxonanes **3h**, **4h**, **5h**, and **6h** were prepared using slightly modified procedures of Hawkins [16] and McCullough [3] using 50% rather than 86% H_2O_2; in the case of hexaoxonane **7h**, half the quantity of sulfuric acid was employed. Hexaoxonanes **8h**, **9h** and **10h** were obtained by the above methods developed to prepare tetraoxanes [3,11]. The NMR data for **8h**, **9h** and **10h** have been recently reported [11].

3,3,6,6,9,9-Hexamethyl-1,2,4,5,7,8-hexaoxonane (**1h**).

Compound **1h** was obtained in 73% yield as a colorless solid, mp 90–91 °C (acetonitrile/water, 1:4) (lit. [1b] 97 °C); ^{1}H NMR: δ 1.46 (s, 18H); ^{13}C NMR: δ 21.30, 107.48.

4,7-Dihydroperoxy-4,7-dipropyl-5,6-dioxadecane (**11**).

Compound **1I** was obtained in 35% yield as a colorless solid, mp 51–53 °C (acetonitrile); ^{1}H NMR: δ 0.96 (t, J = 7.3 Hz, 12H), 1.32–1.55 (m, 8H), 1.56–1.82 (m, 8H), 9.60 (s, 2H); ^{13}C NMR: δ 14.25, 17.11, 31.83, 114.50. VPO MW (Chloroform) 287; calcd MW 294.

Anal. Calcd. for $C_{14}H_{30}O_6$: C, 57.12; H, 10.27. Found: C, 57.16; H, 10.01.

3,3,6,6,9,9-Hexapropyl-1,2,4,5,7,8-hexaoxonane (**2h**).

Compound **2h** was obtained in 40% yield as a colorless solid, mp 76–78 °C (ethanol); ^{1}H NMR: δ 0.92 (t, J = 7.0 Hz, 18H), 1.15-1.60 (m, 18H), 1.65-1.90 (m, 6H); ^{13}C NMR: δ 14.34, 17.10, 32.48, 110.67.

Anal. Calcd. for $C_{21}H_{42}O_6$: C, 64.58; H, 10.84. Found: C, 64.64; H, 10.61.

3,3,6,6,9,9-Hexabutyl-1,2,4,5,7,8-hexaoxonane (**3h**).

Compound **3h** was obtained in 32% yield as a colorless solid, mp 62–64 °C (acetonitrile); ^{1}H NMR: δ 0.91 (t, J = 7.0 Hz, 18H), 1.12–1.60 (m, 30H), 1.68–1.86 (m, 6H); ^{13}C NMR: δ 14.02, 22.82, 25.78, 29.77, 110.88. VPO MW (Chloroform) 463; calcd MW 475.

Anal. Calcd. for $C_{27}H_{54}O_6$: C, 68.31; H, 11.47. Found: C, 68.59; H, 11.49.

3,3,6,6,9,9-Hexapentyl-1,2,4,5,7,8-hexaoxonane (**4h**).

Compound **4h** was obtained in 9% yield as a colorless solid, mp 88–90 °C (acetonitrile); ^{1}H NMR: δ 0.89 (t, J = 6.6 Hz, 18H), 1.12–1.60 (m, 42H), 1.68–1.90 (m, 6H); ^{13}C NMR: δ 13.98, 22.49, 23.23, 29.96, 31.88, 110.92. VPO MW (Chloroform) 502; calcd MW 559.

Anal. Calcd. for $C_{33}H_{66}O_6$: C, 70.92; H, 11.90. Found: C, 71.17; H, 11.70.

6,7,15,16,22,23-Hexaoxatrispiro[4.2.4.2.4.2]henicosane (**5h**).

Compound **5h** was obtained in 45% yield as a colorless solid, mp 166–168 °C (acetonitrile) (lit. [14] 166–168 °C); ^{1}H NMR: δ 1.45–1.95 (m, 18H), 2.24 (m, 6H); ^{13}C NMR: δ 24.54, 33.42, 119.19.

7,8,14,15,21,22-Hexaoxatrispiro[5.2.5.2.5.2]tetracosane (**6h**).

Compound **6h** was obtained in 28% yield as a colorless solid, mp 88–90 °C (acetonitrile) (lit. [3] 93–94 °C); ^{1}H NMR: δ 1.35–1.51 (m, 6H), 1.52–1.71 (m, 12H), 1.82 (m, 12H); ^{13}C NMR: δ 22.72, 25.52, 30.64, 107.65.

8,9,17,18,24,25-Hexaoxatrispiro[6.2.6.2.6.2]heptacosane (**7h**).

Compound **7h** was obtained in 15% yield as a colorless solid, mp 108–110 °C (acetonitrile) (lit. [4a] 78–80 °C); ^{1}H NMR: δ 1.30–1.85 (m, 30H), 2.18 (m, 6H); ^{13}C NMR: δ 22.77, 30.02, 32.83, 112.72.

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