Implications for the Existence of a Heptasulfur Linkage in Natural *o*-Benzopolysulfanes

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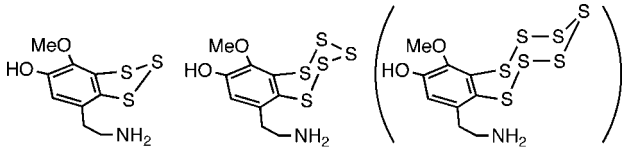
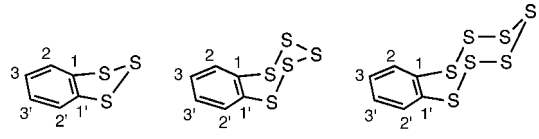
Natural *o*-benzopolysulfanes are often thought to exist as either the trisulfane *or* pentasulfane; the nomenclature has evolved around such notions. No study makes reference to the possible existence of natural *o*-benzoheptasulfanes. The work performed here indicates that a facile equilibration takes place between the tri-, penta-, and heptasulfanes (*o*-$C_6H_4S_3$, *o*-$C_6H_4S_5$, and *o*-$C_6H_4S_7$) in solution. In these simpler (unnatural) compounds, the number of sulfur atoms can be established unequivocally from their independent syntheses. The *o*-benzopolysulfanes, even after purification, yield mixtures of compounds in solution. A similar equilibration may be anticipated for the corresponding natural products.

Determination of the number of sulfur atoms in natural *o*-benzopolysulfanes can be problematic. An example is the cytotoxic material, lissoclinotoxin A, produced by marine invertebrates: in 1991,[1] a prominent MS peak at *m/z* 261 led researchers to conclude that lissoclinotoxin A was a trisulfane (**1**), even though small peaks corresponding to five sulfur (*m/z* 325) and seven sulfur atoms (*m/z* 389) were present.[2] In 1994, it was suggested that the *m/z* 261 fragment peak had been mistaken for the parent peak, and the structure assignment of lissoclinotoxin A was revised to be a pentasulfane possessing 5 sulfur atoms (**2**).[3] The existence of lissoclinotoxin A as the heptasulfane (**3**) has not been suggested despite the mass evidence of the *m/z* 389 peak.[2]



1 **2** **3**
structure not suggested

Such analytical difficulties could be associated with the purity of the isolated materials. In solution, neutral elemental S_8 can form from the natural *o*-benzopolysulfanes via equilibration, even after purification.[4] Computations have been carried out with benzopolysulfanes, *o*-$C_6H_4S_x$ ($x = 1-8$), that are structurally similar to the natural *o*-benzopolysulfanes. In 2004, a density functional theory (DFT) study revealed an interesting stability alternation pattern in these materials.[5] The odd-membered *o*-$C_6H_4S_x$ rings (except $x = 1$ which suffers from ring strain) have enhanced conformational stability compared to the even-membered rings. Thus, *o*-$C_6H_4S_3$ (**4**), *o*-$C_6H_4S_5$ (**5**), and *o*-$C_6H_4S_7$ (**6**) were predicted to be the most stable in the series.



4 **5** **6**

The above mass spectrometry and DFT computed data taken together raise the possibility of the existence of the heptasulfur linkage in natural *o*-benzopolysulfanes and point out the need for additional studies. Here, we present experimental data examining equilibrium patterns among *o*-$C_6H_4S_x$ polysulfanes ($x = 3$, 5, and 7). In these simpler compounds, the number of sulfur atoms can be established unequivocally. Unlike the natural *o*-benzopolysulfanes, the syntheses of **4**–**6** are known by independent methods. In 2004,[5] **4** was observed in a reaction mixture of *n*-BuLi with *o*-dihalobenzene followed by the addition of elemental sulfur, or **4** can be generated by the reaction of 1,2-benzodithiol with dimethyltin dichloride followed by the addition of thionyl chloride ($SOCl_2$) and NaI;[6] in 1984,[7] **5** was generated from the reaction of 1,2-benzodithiolate dianion, *o*-$C_6H_4(S^-)_2$, with sulfur monochloride; and in 2000,[8] **6** was generated from the reaction of 1,2-benzenebis (sulfenyl chloride), *o*-$C_6H_4(SCl)_2$, with titanocene pentasulfide (Cp_2TiS_5). Regardless of the purity achieved via the independent syntheses of **4**–**6** as solids or oils, we provide evidence that, in solution, mixtures of polysulfanes subsequently emerge over time, typically 1–3 days.

Heptasulfane **6** forms via equilibration of elemental S_8 with *o*-$C_6H_4(SH)_2$, or **4** or **5**. Amounts of *o*-$C_6H_4(SH)_2$ (0.15 mM) and S_8 (1.0 mM) were stirred in CH_2Cl_2 at room temperature for 1 day. CH_2Cl_2-insoluble material was removed by filtration. CH_2Cl_2-soluble material was subjected to single ion mode (SIM) GC/MS and HPLC and showed the production of the odd-numbered structures **4**–**6** (Figure 1). In addition, low-resolution GC/MS showed molecular ion peaks corresponding to **4** (*m/z* obsd 172), **5** (*m/z* obsd 236), and **6** (*m/z* obsd 300). Support for the GC/MS and HPLC peak assignments came from spectroscopic comparisons of **4**–**6** each independently synthesized[6−8] and examined shortly after their purification, i.e., before equilibration is pronounced. The identity of the shoulder peak labeled **4′** in Figure 1A is uncertain, but it contains a strong *m/z* 172 peak, which may be due to decomposition of **5** or oligomer structures related to *o*-benzopolysulfanes.[7,9,10] The relative ratios of **4**, **5**, and **6** are 5:82:13 according to the HPLC analysis. The yield of **6** is low, but it demonstrates the viability of the *o*-$C_6H_4(SH)_2$–elemental S_8 reaction in generating the odd-membered benzopolysulfanes **4**–**6**. The SIM-GC/MS analysis facilitated the observation of the weak parent peak of **6**. No evidence was found for the even-numbered sulfur homologues *o*-$C_6H_4S_x$ ($x = 2$, 4, 6, and 8). This result may not be surprising since, as already noted, previous DFT results predicted enhanced conformational stability for the odd-membered *o*-$C_6H_4S_x$ rings ($x = 3$, 5, and 7).[5] The ratio of **4**–**6** is probably solvent-dependent, but it was not examined. There is a possibility of trapping diatomic sulfur in the equilibrium process and that the S_2 unit may be lost and gained by the different odd-numbered polysulfanes involved. Further study is underway to investigate this possibility.[11]

We examined the equilibrium patterns of **4**–**6** (each synthesized and then purified by preparative TLC or centrifugal preparative

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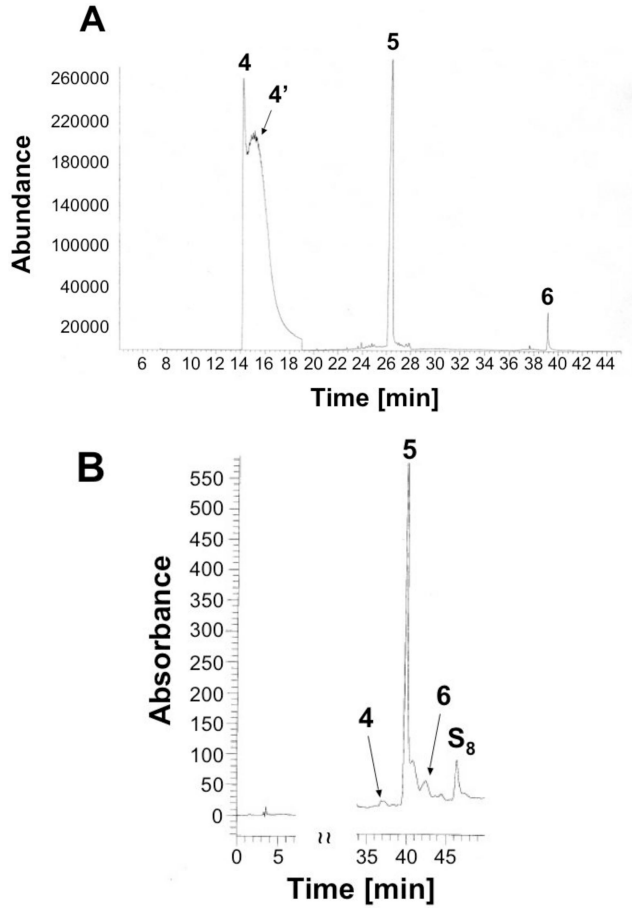

Figure 1. (**A**) Single ion mode (SIM) GC/MS analysis of the thermodynamic equilibrium mixture of *o*-$C_6H_4(SH)_2$ and S_8 in CH_2Cl_2. See text for a discussion of the side peak labeled **4′**. (**B**) HPLC analysis of the thermodynamic equilibrium mixture of *o*-$C_6H_4(SH)_2$ and S_8 in acetonitrile/water mixtures (ranging from 1:9 to 1:0). The absorbance was measured at 256 nm. The additional peaks in the spectrum may correspond to allotropic forms of elemental sulfur based on HPLC analyses of elemental sulfur in the absence of organic polysulfanes.

chromatography) and found similar results to the reaction of *o*-$C_6H_4(SH)_2$ with elemental S_8. Namely, **4** synthesized by the Sato method,[6] upon purification, precipitated S_8 and equilibrated in CH_2Cl_2 over 1 day at room temperature to give 16% **5** and 1% **6**. In CH_2Cl_2, precipitation of S_8 is also observed after the purification of **5** (synthesized by the Chenard method[7]). Here, evidence exists for the involvement of **4** (49%) and **6** (6%) in the equilibration of **5**. By the same token, in CH_2Cl_2, equilibration of **6** (generated by the Steudel method[8] and purified) leads to the production of **4** and **5** (~10–25% total) after 3 days at room temperature. Evidence supporting the formation of mixtures of **4**–**6** in the above reactions is provided by GC/MS and HPLC.

The available inorganic S_8 (which precipitates from *o*-benzopolysulfanes in solution) probably plays a role in determining what structures are favored in the highest proportions. For example, when **4** is purified, it precipitates S_8 in solution, but to a lesser degree compared to the more sulfur rich *o*-benzopolysulfanes **5** and **6**. Thus, heptasulfane **6** forms via equilibration in higher amounts from **5** than from **4** (cf. 6% to 1%). The MS data do not reveal whether the elemental sulfur that precipitates from **4**–**6** takes another form, such as monoclinic or polymeric. The GC/MS retention times of **4** and **5** differ by 12.3 min. The GC/MS retention times of **5** and **6** differ by 12.6 min. We note that **6** gives a weak parent MS peak by GC/MS; however, **6** can be seen clearly by HPLC with the aid of knowing the retention time from its independent synthesis. Therefore, care must be taken in its detection, which may explain why natural *o*-benzoheptasulfanes have not been observed.

In conclusion, it is striking that natural *o*-benzopolysulfanes are often thought to exist as either the trisulfane *or* pentasulfane; the nomenclature has evolved around such notions. No study makes reference to the possible existence of natural *o*-benzoheptasulfanes. The work performed here indicates a facile equilibration takes place between tri-, penta-, and heptasulfanes, **4**–**6**, in solution. This illustrates a key point, namely, that *o*-benzopolysulfanes, even after purification, can yield mixtures of compounds in solution. A similar equilibration may be anticipated for the corresponding natural products.

Experimental Section

General Experimental Procedures. MS data were obtained on an Agilent Technologies 6890N GC/MS instrument. An HP-5 5% diphenyl-95% dimethyl 7 polysiloxane [30 m × 0.25 mm × 0.25 μm (length × inside diameter × film thickness)] fused silica column was used. The GC temperature was ramped using the following program: initial oven temperature 80 °C for 5 min, 15 °C/min to 150 °C and held for 10 min, 30 °C/min to 200 °C and held for 15 min; 30 °C/min to 250 °C and held for 40–50 min. The GC/MS retention times of **4**, **5**, and **6** were 14.2, 26.5, and 39.1 min, respectively. The injector and detector temperatures were held at 275 and 250 °C, respectively. Polysulfide decomposition on the GC/MS injection port has been noted previously.[10,12] HPLC was performed on a Perkin Elmer instrument equipped with an LC 250 pump, a C_{18} column, and a diode array detector. The HPLC analyses were conducted with a flow rate 1 mL/min, which began at an acetonitrile–water ratio of 9:1 and ended at an acetonitrile–water ratio of 1:0 over a 57 min period. The HPLC retention time of **4** was 32.5 min, and for **5** and **6**, the retention times were 39.4 and 43.1 min, respectively. Comparison of integrated peaks of the compounds provided relative percent yields in the HPLC studies, where the polysulfanes **4**–**6** are assumed to possess similar extinction coefficients. NMR spectra were obtained on a Brucker DPX NMR spectrometer. Proton NMR data were acquired at 400 MHz and ^{13}C NMR data were acquired at 100.6 MHz. Purification of polysulfane mixtures were carried out using silica gel 60F 254 TLC plates or by centrifugal (chromatotron) chromatography using Sorbent 60 Å silica gel with gypsum. Reagents and solvents were obtained commercially [1,2-benzodithiol, elemental S_8, CH_2Cl_2, dimethyltin dichloride, CCl_4, $CHCl_3$, $CDCl_3$, thionyl chloride, NaI, $HClO_4$, sodium metal, solid ammonium chloride, sulfur monochloride, sodium bicarbonate, sodium chloride, magnesium sulfate anhydrous, hexanes, carbon disulfide, titanocene dichloride, iron sulfide, sulfuric acid, and ammonia gaseous] and used as received. THF (anhydrous) was distilled over sulfuric acid and stored in the bottle containing sodium metal.

Synthesis of Polysulfanes 4–6. Mixtures of polysulfanes **4**–**6** are generated by the addition of elemental S_8 (1.0 mM) to 1,2-benzodithiol (0.15 mM) in CH_2Cl_2. Elemental sulfur is sparingly soluble in organic solvents.[13] The heterogeneous reaction was stirred at room temperature for 1 day, until 1,2-benzodithiol was not longer detected according to ^1H NMR and GC/MS. The solid material in the reaction was filtered off, and the CH_2Cl_2-soluble portion was analyzed by GC/MS and HPLC. In addition to the detection of **4**–**6**, evidence was found for the presence of other polysulfanes, such as tetrathiocin, or related dimeric or oligomeric compounds, but low yields or low solubility precluded their structure determination. The even-numbered *o*-$C_6H_4S_x$ rings ($x = 2$, 4, 6, and 8) are not generated easily in the reactions; after careful analysis, no evidence could be found for their existence. The HPLC results demonstrated that **6** is present in the reaction and does not arise from a different polysulfane structure via the use of electron impact mass spectrometry. The *o*-$C_6H_4(SH)_2$–S_8 reaction is useful for the formation of mixtures of **4**–**6**. Support for the structures assigned

Table 1. NMR Spectroscopic Data for Polysulfanes **4–6**

	trisulfane (**4**)			pentasulfane (**5**)			heptasulfane (**6**)		
position	δ_C	δ_H (*J* in Hz)	ref	δ_C	δ_H (*J* in Hz)	ref	δ_C	δ_H (*J* in Hz)	ref
1,1′	144.0, C			144.1, C			138.1, C		
2,2′	136.2, CH	7.73, dd (8,4)		136.2 CH	7.83, m		135.5, CH	7.74, m	
3,3′	130.2, CH	7.43, dd (8,4)		130.2, CH	7.33, m		131.9, CH	7.45, m	
				143.8, C		8	137.9		8
				135.8, CH	7.85, m		135.2, CH	7.77, m	
				129.6, CH	7.36, m		131.3, CH	7.48, m	
					7.85–7.77	7			
					7.45–7.2 (AA′BB′)				
					7.52 centered (AA′BB′)	14			
				144.1, C		5			
				136.1, CH	7.85, dd (6, 3)				
				130.2, CH	7.34, dd (6, 4)				

[a] This work.

to **4–6** in the *o*-$C_6H_4(SH)_2$–S_8 reaction came from the independent synthesis of **4–6** by literature procedures as summarized in Table 1.[6–8]

Polysulfanes **4–6** were synthesized individually by literature procedures (Table 1). Because polar solvents can promote the formation of polysulfane mixtures, purification of individual samples of **4**, **5**, or **6** was conducted with CCl_4 or hexanes:$CHCl_3$ (98:2). We prepared *o*-$C_6H_4S_3$ **4** by the method of Sato et al.[6] During the course of the synthesis, intermediates benzodithiastannole and benzotrithiole-2-oxide were purified by preparative TLC (CCl_4:$CHCl_3$, 1:1). Compound **4** was purified by preparative TLC using CCl_4, and was obtained as a light yellow solid after preparative TLC. *o*-$C_6H_4S_5$ **5** was prepared using the method of Chenard et al.,[7] where **5** was purified by centrifugal (chromatotron) chromatography (hexanes:CH_2Cl_2, 98:2). *o*-$C_6H_4S_7$ **6** was synthesized as a viscous yellow liquid with the known procedure of Steudel et al.[8] Purification was accomplished by preparative TLC (hexanes:CH_2Cl_2, 98:2). The spectroscopic assignments of **4–6** are clear; however, in solution, the purity of these individual samples decreased over time, ~1–3 days. Examination of impurities, such as thiolate ions or other nucleophiles, and their possible influence on the equilibration of the initially pure samples was not conducted. A control reaction was conducted to mimic the high temperature injection conditions of GC/MS. A sample containing **4–6** and elemental sulfur when heated to 260 °C decomposed forming black solid material. After cooling, this solid appeared to dissolve in ethyl acetate and yielded broad GC/MS peaks of low sensitivity to detection corresponding to decomposed material. We neither observe additional 16 amu fragment peaks suggesting autoxidation of the heated polysulfane sample nor peaks corresponding to *o*-benzopolysulfanes or other polysulfanes.

1,2,3-Benzotrithiole (4): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.73 (2H, dd $J = 8$, 4 Hz), 7.43 (2H, dd $J = 8$, 4 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 144.0 (2C, C-1 and C-1′), 136.2 (2C, C-2 and C-2′), 130.2 (2C, C-3 and C-3′). EIMS *m/z* 172 $[M]^+$ (100), 140 (5), 128 (2), 108 (64), 96 (13), 82 (7). Literature values of **4** are as follows:[5] Previous NMR data was not available. EIMS *m/z* 172 $[M]^+$ (99), 140 (4), 128 (3), 108 (100), 96 (21), 82 (14), 74 (4), 69 (42), 64 (15), 51 (5).

5,6,7,8,9-Pentathiabenzocycloheptene (5): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.83 (2H, m), 7.33 (2H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 144.1 (2C, C-1 and C-1′), 136.2 (2C, C-2 and C-2′), 130.2 (2C, C-3 and C-3′). EIMS *m/z* 236 $[M]^+$ (14), 172 (100), 140 (17), 108 (29), 96 (16), 82 (4). Literature values of **5** are as follows:[7] ^{1}H NMR δ 7.85−7.77 and 7.45−7.2 (AA'BB′). Steudel et al. also reported spectroscopic data for **5**:[8] ^{1}H NMR (CD_2Cl_2, 200 MHz) δ 7.85 (2H, m), 7.36 (2H, m); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 143.8 (2C), 135.8 (2C), 129.6 (2C). EIMS *m/z* 236 $[M]^+$ (16), 172 (100).

5,6,7,8,9,10,11-Heptathiabenzocyclononene (6): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.74 (2H, m), 7.45 (2H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 138.1 (2C, C-1 and C-1′), 135.5 (2C, C-2 and C-2′), 131.9 (2C, C-3 and C-3′). EIMS *m/z* 300 $[M]^+$ (4), 268 (5), 172 (100), 108 (59). Literature values of **6** are as follows:[8] ^{1}H NMR (CD_2Cl_2, 200 MHz) δ 7.77 (2H, m), 7.48 (2H, m); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 137.9 (2C), 135.2 (2C), 131.3 (2C). EIMS *m/z* 300 $[M]^+$ (2), 172 (100).

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