SYNTHESIS OF 4,4'-BIS([^{3}H]METHYLSULPHONYL)-2,2',5,5'-TETRACHLOROBIPHENYL

E. Klasson-Wehler, Å.L. Bergman and C.A. Wachtmeister

Section of Organic Chemistry
Wallenberg Laboratory
University of Stockholm
S-106 91 STOCKHOLM, Sweden

SUMMARY

Tritium labelled 4,4'-bis(methylsulphonyl)-2,2',5,5'-tetrachlorobiphenyl with tritium in one or both methyl groups was synthesized. Sequential nucleophilic substitutions of 2,2',4,4',5,5'-hexachlorobiphenyl with methane thiolate and sodium hydrogen sulphide respectively, gave 4'-methylthio-2,2',5,5'-tetrachloro-4-biphenylthiol which subsequently was methylated with [^{3}H]methyl iodide and oxidized. 2,2',5,5'-Tetrachlorobenzidine was diazotized and coupled with potassium O-ethyl dithiocarbonate. The product was reduced, methylated and oxidized to the sulphone with uniformly labelled methyl groups.

INTRODUCTION

Technical polychlorobiphenyl (PCB) mixtures such as Aroclor 1016, 1242 and Clophen A30, A40, A50 and A60 contain more than fifty different chlorinated biphenyls. 2,2',5,5'-Tetrachlorobiphenyl (tetraCB) is one of the biphenyls present in these PCB mixtures (1,2), and it has also been detected in human adipose tissue (3).

The metabolism of tetraCB has been extensively studied and a number of metabolites have been identified. The tetraCB metabolism in mammals was recently summarized by Bergman *et al.* (4), who also reported on the formation of a bis-(methylsulphonyl)-tetraCB metabolite in mouse. The corresponding bis(methylthio)-tetraCB was synthesized labelled with carbon-14 (5) and the tissue residue distribution studied by autoradiography in mice (6). This sulphide gave rise to an extraordinary retention of radioactivity in the lung bronchial mucosa and kidney cortex. It is known that polychlorobiphenyls of certain structures are transformed into methyl sulphone metabolites that accumulate in these sites (7,8).

0362-4803/83/121407-06$01.00

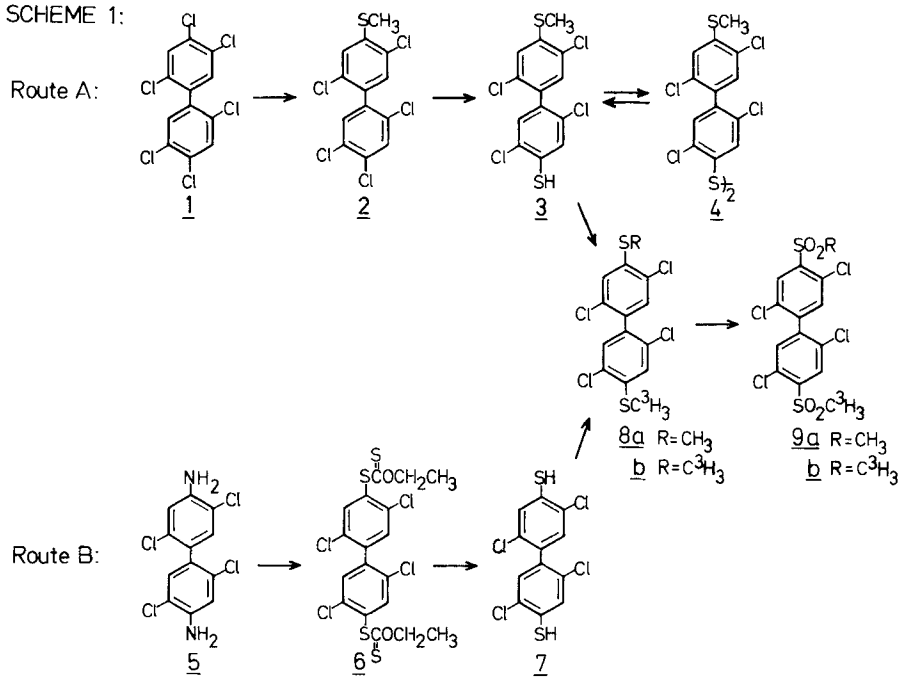
Received August 8, 1983
Revised September 20, 1983

In order to determine more exactly the site(s) of accumulation of 4,4'-bis-(methylsulphonyl)-tetraCB, the expected major accumulating agent, using micro-autoradiographic and receptor studies, bis([^{3}H]methylsulphonyl)-tetraCB was synthesized. The present paper describes two routes for its preparation.

RESULTS AND DISCUSSION

Chlorine atoms in certain positions of aromatic compounds, such as polychlorinated biphenyls (5) and hexachlorobenzene (9), have been shown to be substituted by methane thiolate. Thus 2,2',4,5,5'-pentachlorobiphenyl gave mainly 4-methylthio-2,2',5,5'-tetrachlorobiphenyl whereas 2,2',4,4',5,5'-hexachlorobiphenyl gave both 4-methylthio-2,2',4',5,5'-pentachlorobiphenyl and 4,4'-bis-(methylthio)-2,2',5,5'-tetrachlorobiphenyl (5). Results reported by Testaferri et. al. (10) indicate that alkylthiolates in hexamethyl phosphoramide (HMPA) readily replaced aromatic halogen atoms. Therefore, with 2,2',4,4',5,5'-hexachlorobiphenyl,1, using sodium hydrogen sulphide in HMPA as the nucleophile, 2,2',5,5'-tetrachloro-4,4'-biphenyldithiol 7 was expected to be formed. Formation of 7 was determined by comparison of TLC R_f values and GC retention times of the diazomethane treated reaction product of 7 and the authentic reference methyl sulphide. Only minor amounts of the dithiol were however isolated because of unfavorable partitioning properties between HMPA/water and organic solvent and because of rapid oxidation to products not reduced by lithium aluminium hydride. A modified route (route A, Scheme 1) and a Leuckart thiophenol reaction route (route B, Scheme 1) for the synthesis of 7 were therefore devised.

Route A started with synthesis of 2,2',4,4',5,5'-hexachlorobiphenyl,1, which was prepared as described by Sundström (11). The hexachlorobiphenyl 1 was treated with methane thiolate in methanol (5) and 4-methylthio-2,2',4',5,5'-pentachlorobiphenyl 2, was obtained as the major product. In the next step this sulphide was allowed to react with sodium hydrogen sulphide under conditions described by Testaferri et. al. (10). A major product, isolated via the corresponding disulphide, was identified as 4'-methylthio-2,2',5,5'-tetrachloro-4-biphenylthiol, 3. Identity of the compound was confirmed by comparison of the GC and GC-MS properties of methylated 3 with authentic 4,4'-bis(methylthio)-2,2',5,5'--tetraCB (5). Compound 3 was methylated with [^{3}H]methyl iodide and subsequently oxidized with m-chloroperbenzoic acid (mCPBA).The methylation reaction was performed in the methyl iodide ampoule. The ampoule was connected to a cold trap containing potassium O-ethyl dithiocarbonate dissolved in acetone to trap unreacted methyl iodide.



The starting material for route B, 2,2',5,5'-tetrachlorobenzidine 5, was diazotized and reacted with potassium O-ethyl dithiocarbonate (12) to give 4,4'-bis-[ethoxy(thiocarbonylthio)]-tetraCB 6, which was purified by liquid chromatography. The ester 6 was reduced (13) and the dithiol 7 was immediately methylated as described above, in order to avoid losses of thiol by disulphide formation. The methyl sulfide 8b was purified and oxidized with mCPBA in chloroform.

MATERIALS AND METHODS

[^{3}H]Methyl iodide (Amersham International plc.), 2,2',5,5'-tetrachlorobenzidine (Pfaltz & Bauer), sodium hydrogen sulphide monohydrate (Fluka), potassium O-ethyl dithiocarbonate (Fluka). Silica gel 60, <0.063 μ (Merck) was used for liquid chromatography. Low pressure liquid chromatography (LPLC) was run on a LiChroprep RP-8 (40-60 μ) glass column (Merck) with an Altex 100 pump. High pressure liquid chromatography (HPLC) was performed on a RP-8 column on line with a Packard TriCarb 7500 radioactivity monitor (RAM). Thin layer chromatography (TLC) was performed on silica gel plates (Kieselgel 60F-254, 0.25 mm, Merck). GC-MS was run on a Finnigan 4021 mass spectrometer. ^{1}H NMR were ob-

tained, in $CDCl_3$ with TMS as internal reference standard unless otherwise stated, on a Jeol FX-100 instrument. Radioactivity measurements were performed on a Packard Tri-carb 460 C scintillation counter with samples dissolved in toluene PPO/POPOP.

4'-Methylthio-2,2',5,5'-tetrachloro-4-biphenylthiol, 3:
2,2',4,4',5,5'-Hexachlorobiphenyl was synthesized from 2,4,5-trichloroiodobenzene as described (11). Compound 2 was isolated as a major product from the hexachlorobiphenyl reaction with methane thiolate in methanol (5). Sodium hydrogen sulphide monohydrate (2.2 g, 30 mmol) was added to a solution of 2 (1.0 g, 2.7 mmol) in hexamethyl phosphoramide (70 ml) and the reaction mixture was stirred at 60°C under a continuous flow of nitrogen for 5 h. The mixture was poured into water, acidified and extracted with ether. The organic phase was filtered and the ether solution was dried over sodium sulphate. The crude product was purified by oxidation to the disulphide 4, with iodine in ethyl acetate (30 ml, 0.13 M). After 30 min at room temperature, sodium thiosulphate (15 ml, 0.6 M) was added. The ethyl acetate was evaporated and the remaining water phase, containing a white precipitate, was extracted with chloroform. The disulphide was obtained pure in 96% yield from a silica gel (200 g) column with hexane:chloroform (4:1) as the mobile phase. Disulphide 4 (16 mg, 21 μmol) in dry ether (5 ml) was reduced prior to the methylation procedure, with lithium aluminium hydride (15 mg, 0.4 mmol) under reflux for 1.5 h. The thiol was obtained in quantitative yield after extraction with ether. ^{1}H NMR of thiol 3: δ (ppm) 7.46 (s, 2H), 7.22 (s, 1H), 7.20 (s, 1H), 4.01 (s, 1H) and 2.52 (s, 3H). GC-MS (70 eV): m/z 368 (M^+, 100%), 353 $(M-CH_3)^+$, 333 $(M-Cl)^+$, 318 $(M-(CH_3Cl)^+$.

4,4'-bis([^{3}H]methylthio)-2,2',5,5'-tetrachlorobiphenyl, 8a:
The thiol, 3 (8 mg, 21.6 μmol) was dissolved in acetone (1 ml) and added to cooled [^{3}H]methyl iodide (925 MBq, 481 GBq/mmol) in the original ampoule. Sodium hydrogen carbonate (1 mg), suspended in acetone (0.5 ml), was added to the mixture, which then was refluxed for 4 h. The ampoule was connected to a dry ice trap containing potassium O-ethyl dithiocarbonate (40 mM in acetone). The reaction was stopped and the solvent and remaining [^{3}H]methyl iodide were evaporated under a flow of nitrogen. The residue was dissolved in chloroform and the crude product purified by TLC (mobile phase; hexane:chloroform, 9:1). The 4,4'-bis(methylthio)-tetraCB containing TLC-band was isolated.

4,4'-bis([^{3}H]methylsulphonyl)-2,2',5,5'-tetrachlorobiphenyl, 9a:
The sulphide 8a (166 MBq, 481 GBq/mmol), in chloroform (5 ml), was oxidized to the corresponding sulphone with m-chloroperbenzoic acid (100 mg, 0.57 mmol). After stirring overnight at room temperature, benzoic acid and remaining per-

benzoic acid were separated from 9a on an alkaline aluminium oxide (5 g) column with chloroform as the mobile phase. The sulphone 9a (155 MBq, 481 GBq/mmol) was isolated after TLC purification (hexane:chloroform, 1:1). ^{1}H NMR of unlabelled 9: (in DMSO-d_6) δ (ppm) 8.16 (s, 2H), 8.02 (s, 2H), 3.33 (s, 6H). Mass spectrum of unlabelled 9a was in accordance with the spectrum of 4,4'-bis(methylsulphonyl)-2,2',5,5'-tetrachlorobiphenyl published elsewhere (5). Only one peak was determined by HPLC-RAM, containing 90% of the total activity injected on the column.

4,4'-Bis[ethoxy(thiocarbonylthio)]-2,2',5,5'-tetrachlorobiphenyl, 6:
2,2',5,5'-Tetrachlorobenzidine (250 mg, 0.8 mmol) was dissolved in conc. sulphuric acid (2.5 ml) and placed in an ice bath. The acidic solution was carefully diluted with water (15 ml). The amine was diazotized with sodium nitrite (4.0 ml, 0.43 M). Potassium O-ethyl dithiocarbonate (582 mg, 3.6 mmol) was dissolved in water (20 ml) and nickel(II)sulphate (10 mg) was added as a catalyst (14). The cold diazonium salt solution was added to the potassium O-ethyl dithiocarbonate solution (< 5°C) in portions. The temperature of the reaction mixture was allowed to increase to room temperature overnight. The reaction mixture was extracted with ether and transferred to a silica gel (30 g) column (mobile phase; hexane:ethyl acetate, 9:1). A fraction containing two compounds was collected and further purified by LPLC with methanol as mobile phase. A yield of 25% of compound 6 was obtained. ^{1}H NMR of 6: δ (ppm) 7.52 (s, 2H), 7.38 (s, 2H), 4.76 (q, 7.1Hz, 4H) and 1.50 (t, 7.1Hz, 6H).

2,2',5,5'-tetrachloro-4,4'-biphenyldithiol, 7:
Compound, 6, (1.2 mg, 2.3 μmol) was dissolved in dry ether (3.5 ml), lithium aluminium hydride (6 mg, 0.16 mmol) was added and the mixture was refluxed for 1.5 h. Sulphuric acid (0.5 ml, 1.9M) and water (1 ml) were added carefully and the mixture was extracted with ether. The organic phase was dried over sodium sulphate, concentrated and the thiol 7 was dissolved in acetone (2 ml). One half was methylated with diazomethane and quantified by GC (the yield was quantitative). ^{1}H NMR of 7: δ (ppm) 7.45 (s, 2H), 7.25 (s, 2H) and 4.01 (s, 2H). The other half was immediately methylated with [^{3}H]methyl iodide.

4,4'-bis([^{3}H] methylsulphonyl)-2,2',5-5'-tetrachlorobiphenyl, 9b:
The thiol 7 was methylated with [^{3}H]methyl iodide (925 MBq, 296 GBq/mmol) as described above and the sulphide was oxidized with mCPBA. The yield of 9b was 185 MBq (592 GBq/mmol) and with the corresponding radiochemical purity as 9a.

ACKNOWLEDGMENT

Financial support was given by the Swedish Council for Planning and Coordination of Research.

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