Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXXVI, No. 9

SYNTHESIS OF MIXED HALOGENATED TRIHALOMETHANES LABELLED WITH THE STABLE ISOTOPES ^{2}H OR ^{13}C

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SUMMARY

^{2}H and ^{13}C-labelled bromodichloromethane and chlorodibromomethane were obtained in yields of 20 and 12% (for ^{2}H) and 6 and 3% (for ^{13}C) respectively from a convenient aluminum chloride-catalyzed reaction of bromoethane with commercially-available isotopically-substituted chloroform. The products were purified by preparative gas chromatography and characterized by MS and ^{13}C NMR, and by ^{1}H for the ^{13}C-labelled material; mass and nuclear magnetic resonance spectroscopy data are reported.

Key words: Trihalomethanes, [^{2}H]bromodichloromethane, [^{13}C]bromodichloromethane, [^{2}H]chlorodibromomethane, [^{13}C]chlorodibromomethane

INTRODUCTION

Trihalomethanes (THMs), which have been found in chlorinated drinking waters at concentrations from 0.1 to 540 μg/L, are currently regulated in the U.S.A. at 100 μg/L (1). Generally the most abundant THM is chloroform ($CHCl_3$), a suspect human carcinogen. When waters high in bromide are chlorinated, the brominated THMs bromodichloromethane ($CHBrCl_2$), dibromochloromethane ($CHBr_2Cl$) and bromoform are formed (2). $CHBrCl_2$ appears to be more carcinogenic than $CHCl_3$ in rodent bioassays (3,4), and also more toxic (5). Less is known of the toxicological properties of

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CCC 0362–4803/95/090835–09

Received 19 December 1994
Revised 13 March 1995

$CHBr_2Cl$. The potential health implications of widespread population exposure to the mixed halogenated THMs have stimulated efforts to explore the relationship between their structure, metabolism, and different toxic endpoints. Stable isotopes represent a useful tool for these studies, both for mechanistic determinations (such as deuterium isotope effect on metabolic C-H bond cleavage) and as tracers (^{13}C). In support of such studies (6), we undertook preparation of isotopically-labelled mixed halogenated trihalomethanes that are not currently available commercially.

Syntheses of $CHBrCl_2$ and $CHBr_2Cl$ have been reported previously (7, 8). One approach (7) would have required both labelled chloroform and bromoform, and yielded undesirable by-products. The other (8) was applicable only to deuterated materials; the reported extent of deuterium incorporation (36% for $CHBrCl_2$, 64% for $CHBr_2Cl$) was insufficient for our purposes. A more promising approach was suggested by a reported synthesis of $CHBrCl_2$ in 35% yield through halogen exchange between $CHCl_3$ and bromoethane (C_2H_5Br) in the presence of catalytic amounts of aluminum chloride, with purification by fractional distillation (9).

$$CHCl_3 + C_2H_5Br \xrightarrow{AlCl_3} CHBrCl_2 + C_2H_5Cl$$

Because of the absence of complex by-products and the convenient separation of desired products, this approach was modified for synthesis of ^{2}H and ^{13}C-labelled bromodichloromethane, starting from commercially-available, appropriately isotopically-substituted, chloroform. Proper scaling of the reaction, and efficient purification and recovery of product, were all essential to achieve optimal use of the labelled starting materials. The procedure developed would also be readily applicable for preparation of radioisotopes.

EXPERIMENTAL

Instrumentation: ^{13}C nuclear magnetic resonance (NMR) spectra were recorded at room temperature in deuterated acetone on a model XL-400 NMR

spectrometer (Varian Instrument Group., Walnut Creek, CA) operated at 100.573 MHz, with spectral width 22.6 kHz, pulse angle 39°, and gated proton decoupling. ^{1}H-NMR spectra were obtained in deuterated acetone at 500 MHz on a Brucker AMX500 (Brucker Instrument Co., Billerica, MA). Chemical shifts are reported in ppm relative to TMS. GC-MS spectra were acquired with a gas chromatograph (Model 5890 Series II, Hewlett Packard, Roseville, CA) fitted with a 30 meter DB-5 column, 0.242 mm id and 1 mm phase thickness (J & W Scientific, Folsom, California) interfaced to a Hewlett Packard 5971A Mass Selective Detector operated at full scan 50 - 550 amu in the EI mode at 70 eV. Preparative GC separations were performed on a Varian 920 Aerograph gas chromatograph equipped with a thermal conductivity detector and a 1% AT-1000 on Graphpac GB 60/80 1/4" by 5" stainless steel column (Alltech Associates, Deerfield, IL).

Materials: $CDCl_3$, with isotopic purity 99.8%, was obtained from Isotec Inc. (Miamisburg, OH). $^{13}CHCl_3$, chemical purity > 98% and isotopic purity 99%, was purchased from Cambridge Isotope Laboratory (Woburn, MA). Bromoethane was from Eastman Kodak (Rochester, NY). All other chemicals were obtained at the highest degree of purity available commercially.

Analytical gas chromatography/mass spectrometry: The carrier gas was helium, flow rate 1 mL/min. The injector temperature was 150°C and the detector temperature was 280°C. Samples diluted in CH_2Cl_2 (2.5 μL/mL) were injected (1 μL) at a split ratio of 1:30. The column temperature was held at 50°C for 5 min. Data acquisition was started after a solvent delay of 1 min, to enable detection of low-boiling constituents. Column temperature was programmed at 10°C/min to 150°C, then held for 5 min. For quantitation, areas of total ion current peaks were integrated (proprietary software HPChemStation Vers. B, 1990, Hewlett-Packard). Mass spectra were averaged across the peak at half-height.

Preparative gas chromatography: The carrier gas was helium, at 60 mL/min and the injector was not heated. Samples were injected neat on-column (0.2

mL for the [^{13}C]THMs, 0.5 mL for the deuterated THMs) at 70°C. The oven temperature was raised under manual control to 110°C while C_2H_5Br and C_2H_5Cl eluted, to 130°C while $CHCl_3$ eluted, to 150°C while $CHBrCl_2$ eluted, to 170°C while $CHBr_2Cl$ eluted, then to 200°C. The thermal conductivity detector was operated at 150 mA. Detector temperature was 170°C. The CG effluent was directed through a vertical 10-cm ice-water-cooled condenser (fabricated in-house) connected to the GC outlet port by a glass-to-metal ball-and-socket joint. Fractions were collected based on the detector signal and the appearance of liquid droplets in the condenser into individual collection vials connected to the condenser with a ground glass joint and cooled by immersion in a dry ice-acetone bath. Vials were changed manually between eluting peaks. Uncondensed effluent was directed through a T-joint at the base of the condenser to a secondary trap cooled with an acetone/dry ice bath. Separations of a 1:1 mixture of unlabelled $CHCl_3$ and $CHBrCl_2$ achieved 50 to 55 % recovery for $CHCl_3$ and 80 to 85% recovery for $CHBrCl_2$.

Preparation of ^{2}H-Labelled Bromodichloromethane and Chlorodibromomethane: $CDCl_3$ (25 mL) and C_2H_5Br (25 mL) were stirred in a 100 mL round bottom flask, cooled in an ice bath. $AlCl_3$ (approx. 1 g) was added until the reaction mixture turned bright red. The flask was then sealed with an anhydrous $CaSO_4$ drying tube to exclude moisture, and covered with aluminum foil to protect the light-sensitive THMs. Optimum yield of $CDBrCl_2$ was obtained when the reaction mixture was stirred for 46 hours at room temperature.

The reaction was stopped by adding deionized water (15 mL) and 1 M HCl (10 mL). The organic layer was washed with 5% w/v aq. NaOH (30 mL), then dried over sodium sulfate. The solution was distilled through a Vigreux column (12 cm high by 1 cm diameter), and three fractions were collected: fraction 1: 24-64°C; fraction 2: 65-72°C; and fraction 3: 73-78°C. For reference, $CHBrCl_2$ boils at 87°C and $CHBr_2Cl$ at 119-120°C/748 mm (10).

For production of $CDBr_2Cl$, the reaction time was increased to 7 days, and five distillation fractions were collected: fraction 1: 27-67°C;

fraction 2: 68-72°C; fraction 3: 73-78°C; fraction 4: 79-84°C and fraction 5: 90°C (under vacuum). A portion of distillation fraction 4 was further purified by preparative GC.

Preparation of ^{13}C-Labelled Bromodichloromethane and Chlorodibromomethane: For synthesis of the ^{13}C-labelled THMs, the reaction was repeated on a reduced scale (dictated by the cost of the starting material): 5 g (3.3 mL) $^{13}CHCl_3$ and 3.3 mL C_2H_5Br in a 25 mL round bottom flask covered with aluminum foil. $AlCl_3$ was added until the mixture turned bright red. After stirring (sealed with a drying tube) at room temperature for 93 hours, the reaction was stopped by adding deionized water (10 mL) and 1 M HCl (5 mL). The organic layer was washed with 5% (w/v) aq. NaOH and dried over sodium sulfate. The product was purified by preparative GC.

RESULTS AND DISCUSSION

Both $CDCl_2Br$ and $CDBr_2Cl$ were recovered from the halogen exchange reaction of $CDCl_3$ with C_2H_5Br in the presence of $AlCl_3$. These compounds were identified by comparison of their GC retention times (Table 1) and natural abundance ^{13}C NMR spectra (Table 2) to those of unlabelled reference compounds. Retention of the deuterium label was confirmed by the appearance of triplet signals in the ^{13}C NMR spectra. Mass spectra (Table 1) were consistent with the assigned structures. Molecular ions were weak; for both compounds, the predominant ions arose from loss of bromine. Relative intensities for the halogen clusters corresponded to those predicted from theoretical isotope ratios.

In reactions conducted for 46 h, two fractions of interest were recovered. By GC-MS analysis, the fraction boiling at 65-72°C was estimated to contain 93% $CDBrCl_2$, and the fraction boiling at 73-78°C was 85% $CDBrCl_2$. The balance of the later fraction was $CDBr_2Cl$, presumably arising from further halogen exchange with the newly-formed $CDBrCl_2$.

When the duration of the reaction was increased to 7 days, and the distillate collected in tighter cuts, $CDBr_2Cl$ was found in fractions 4 and

Table 1: Mass spectroscopy characteristics of isotopically-substituted trihalomethanes[a].

	R_T (min)	$[M]^+$	$[M-Cl]^+$	$[M-Br]^+$	$[M-X_1-X_2]^+$ [b] + $[M-X_1-X_2-D]^+$	$[M-X_1-X_2-H]^+$
$CDBrCl_2$	4.6	163, 165, 167	128, 130, 132	84, 86, 88	91, 93, 95	-
		(weak, <0.1)	(15, 19, 4)	(100, 71, 11)	(4, 6, 2)	-
$CDBr_2Cl$	7.6	207, 209, 211	172, 174, 176	128, 130, 132	91, 93, 95	-
		(1, 2, 2)	(1, 2, 1)	(73, 100, 19)	(6, 10, 3)	-
$[^{13}C]CHCl_3$	2.5	119, 121, 123	84, 86, 88	-	-	-
		(~1)	(100, 63, 10)	-	-	-
$[^{13}C]CHBrCl_2$	4.6	163, 165, 167	128, 130, 132	84, 86, 88	-	92, 94
		(weak, <0.1)	(16, 21, 5)	(100, 77, 13)	-	(6, 6)
$[^{13}C]CHBr_2Cl$	7.6	207, 209, 211	172, 174, 176	128, 130, 132	-	92, 94
		(1, 3, 2)	(1, 2, 1)	(76, 100, 24)	-	(9, 9)
$[^{13}C]CHBr_3$	10.1	251, 253, 255, 257	-	172, 174, 176	-	92, 94
		(4, 11, 10, 4)	-	(51, 100, 49)	-	(10, 9)

[a] Data are presented as m/z of major ions above 50, with relative intensity in parentheses, determined by GC/MS with a J & W DB-5 capillary column and a Hewlett-Packard Mass Selective Detector.

[b] For bromodichloromethane, $X_1 = X_2 = Cl$; for dibromochloromethane, $X_1 = Cl$, $X_2 = Br$; for bromoform, $X_1 = X_2 = Br$.

Table 2: NMR spectroscopy characteristics of isotopically-substituted trihalomethanes

	^{13}C-NMR[a]		^{1}H-NMR[b]	
	δ_C (ppm)	$J13_{C-D}$ (Hz)	δ_H (ppm)	$J13_{C-H}$ (Hz)
Bromodichloromethane	58.7 (t)	32.7	7.92 (d)	214
Dibromochloromethane	36.7 (t)	32.4	7.76 (d)	212

[a] Data shown are for natural abundance ^{13}C in deuterated THMs analysed by ^{13}C-NMR at 100 MHz in acetone-d_6. Shifts are comparable to literature values (11).

[b] Data shown are for ^{13}C-labelled THMs analysed by ^{1}H-NMR at 500 MHz in acetone-d_6. The ^{13}C-labelled THMs were also analysed by ^{13}C-NMR at 100 MHz in acetone-d_6; their chemical shifts were identical to those of the natural abundance ^{13}C signals, and the ^{13}C-H coupling constants were identical to those measured by ^{1}H-NMR analysis.

5 (20% and 75% respectively, extimated by ^{13}C-NMR), while $CDBrCl_2$ was present in fractions 2-5. Yields were low (Table 3), but acceptable considering the low cost of the deuterated starting material. Further purification of 2 g of Fractions 4 by preparative CG afforded 1.3 g of $CDBrCl_2$ containing less than 0.5% detectable $CDBr_2Cl$. Thus the availability of a preparative GC system greatly facilitates obtaining final products of high purity.

Smaller scale preparations utilizing $^{13}CHCl_3$ were carried out for intermediate durations (90 h) in order to maximize product diversity. $^{13}CHBr_3$ was detected by GC/MS analysis, in addition to the two desired products, the by-product C_2H_5Cl, and unreacted C_2H_5Br and $^{13}CHCl_3$. The latter three compounds would have been collected in the low-boiling fractions (<65°C) from the Vigreux distillation. All four ^{13}C-labelled THMs have mass spectra (Table 1) and ^{1}H-NMR spectra (Table 2) consistent with the assigned structures. The final purity of the $^{13}CHBrCl_2$ was > 99% by both GC and ^{1}H-NMR (by integration of signals), with the level of $^{13}CHBr_2Cl$ less than that of $^{12}CHBrCl_2$. $^{13}CHBr_2Cl$ proved more difficult to rid of the lower-boiling $^{13}CHBrCl_2$; final purity was 89% by GC/MS and 83% by ^{1}H-NMR, with the balance mainly $^{13}CHBrCl_2$.

Fragmentation of the bromine-containing THMs gave a cluster of ions of equal intensity at *m/z* 91 and 93 due to $[CBr]^+$ or *m/z* 92 and 94 due to

Table 3: Product yields from reaction of bromoethane with chloroform in the presence of aluminum chloride[a].

Chloroform Isotope	Product: Bromodichloromethane	Chlorodibromomethane
Deuterium (25 mL, 37.5 g, 46 h)	11.6 g (23%)[b]	1.0 g (2%)
Deuterium (25 mL, 37.5 g, 7 d)	4.0 g (8%)[b]	5.0 g (12%)
^{13}C (3.3 mL, 4.9 g, 93 h)	0.40 g (6%)[c]	0.23 g (3%)

[a] Yields are expressed in g, with percentage yield on a molar basis relative to chloroform in parentheses.

[b] Recoveries based on ^{13}C-NMR analysis of composition of crude fractions collected after Vigreux distillation.

[c] Recoveries after purification by preparative GC.

$[^{13}CBr]^+$, as expected from a monobrominated species. The deuterated THMs however showed a clear and reproducible cluster of ions at *m/z* 91, 93 and 95 with intensities *x*, *x+y*, *y*. This pattern can be explained by retention of deuterium ($[CDBr]^+$), whereas the protiated species readily lost H along with successive halogens to give $[CBr]^+$ exclusively. This feature can serve to distinguish between deuterated and ^{13}C-labelled brominated THMs.

The isotopic enrichment of the final product is identical to that of the starting material. This procedure therefore provides a convenient preparation for isotopically-labelled mixed halogenated THMs that may be of interest for toxicology and analytical studies of disinfection by-products.

ACKNOWLEDGEMENTS

This paper is based on research submitted by H.L. Vermeulen in partial fulfillment of the requirements for the degree of MSPH from the University of North Carolina at Chapel Hill. This work was supported by the U.S. Environmental Protection Agency (EPA) through CR#818558. We thank Dr. P. Albro, NIEHS, for loan of the preparative GC, and Ms K. Yeowell O'Connell and Mr. T. McDonald for help in acquiring GC/MS data. This manuscript has not yet been reviewed in accordance with the policy of the

Health Effects Research Laboratory of the U.S. Environmental Protection Agency and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

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