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^{124}Sn-LABELLED TETRA-n-BUTYLTIN AND TRI-n-BUTYLTIN BROMIDE

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SUMMARY

Stable isotope-labelled tetra-n-butyl(124)tin was synthesized by the reaction of n-butylmagnesium bromide with $^{124}SnCl_4$. Tri-n-butyltin bromide was synthesized by bromine cleavage of the tetraalkyltin. The compound can be used to trace chemical, biological and physical processes in environmental and metabolic studies using gas chromatography and electron impact low resolution mass spectrometry. The simplified spectra of the isotopically enriched compounds leads to a twofold increase in detection sensitivity by GC/MS.

Key Words: Organotins, ^{124}Sn, Tetra-n-butyl(124)tin, Tri-n-butyl(124)tin bromide, stable isotope labelling, GC/MS

INTRODUCTION

Tri-n-butyltin compounds, mainly from use as antifoulant coatings, have caused increased environmental concern. Their environmental fate is not completely understood, and their degradation pathways are uncertain.

Tri-n-butyltin compounds which contain the radioisotopes ^{3}H (1), ^{14}C (2,3), and ^{113}Sn (4,5,6) have been synthesized. Tri-n-butyltin oxide, and some related compounds, labelled with ^{14}C have been used for metabolism and accumulation studies in mammals (7,8,9), in fish (10), and in marine invertebrates (11,12). Sheldon and Slesinger (13) and Barug and Vonk (14) looked at

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complete mineralization of tri-n-butyltin oxide in soil using ^{14}C-labelled compounds. ^{113}Sn-labelled tri-n-butyltin oxide was used for metabolic studies in mice (5) and in fish (15).

Although radioactive isotopes are easy to detect, there are hazards and regulatory complications associated with their use. In addition, in the case of ^{14}C- and ^{3}H-labelled compounds, the detection and localization of radioactivity does not ensure its association with the tin atom. We report here the syntheses of tri-n-butyl(124)tin bromide and its intermediate tetra-n-butyl(124)tin. The compounds were enriched 97.4% in ^{124}Sn.

A low abundance stable isotope at either the heavier or lighter end of the mass range of naturally occurring tin is most desirable as a tracer. Although ^{112}Sn is lower in natural abundance than ^{124}Sn, it is also much more expensive. For this reason, ^{124}Sn was purchased for the synthesis.

These labelled compounds can be used to trace chemical, biological and physical processes in environmental and metabolic studies. To identify these compounds, a mass spectrometer is necessary. The mass spectra of the synthesized compounds and a derivative of tributyltin bromide demonstrate the utility of mass spectrometric detection and identification of these compounds when used in tracer studies.

EXPERIMENTAL

Chemicals

Anhydrous tetrachloro(124)tin was obtained from U.S. Services, Summit, NJ. Florisil, 100/200 mesh, was obtained from Supelco Inc., Bellefonte, PA. Tetra-n-butyltin (98% purity), 2.0 M n-butylmagnesium bromide in diethylether and 2.0 M n-hexylmagnesium bromide in diethylether was purchased from Aldrich Chemical Company, Milwaukee, WI. Tri-n-butyltin bromide was purchased from Alfa Products, Danvers, MA.

Instrumentation

Retention times and mass spectra of synthesized and purchased compounds were obtained with a Hewlett-Packard Model 5890A Gas Chromatograph directly connected to a Hewlett-Packard Model 5970 Mass Selective Detector. A Hewlett-Packard 9000-300 Computer using Model 59970C ChemStation software collected the data. Samples were analyzed using splitless injection onto a 12.5 m by 0.2 mm i.d. Hewlett-Packard HP-1 fused silica capillary

column with 0.33 um coating thickness. Helium carrier gas was used at a head pressure of 40 kPa. The oven was programmed, after an initial 2 minute hold at 50° C, to 230° C at 30° C/min. Injector, transfer line and detector were at 250° C. Masses were scanned between 100 and 450 amu to obtain mass spectra. Detection limits were established using optimal selected ions.

Preparation of tetra-n-butyl(124)tin

Approximately 1 g anhydrous tetrachloro(124)tin which was enriched in ^{124}Sn to 97.4% purity was dissolved in 10 ml hexane. The hexane solution was added dropwise to a threefold excess of 2.0 M n-butylmagnesium bromide in diethylether which was cooled to 0° C in an ice bath. When addition was complete, the reaction mixture was refluxed in a hot water bath for 3 hours. The reaction mixture was again cooled to 0° C and then hydrolyzed with 3% HCl. The separated organic layer was shaken with 5% aqueous KF to precipitate contaminating n-butyltin halides as insoluble fluorides. The organic layer was dried with anhydrous Na_2SO_4, and the solvent and low boiling impurities were removed by vacuum distillation at room temperature. Purity of the product was determined by GC/MS. Yield of the tetra-n-butyltin was approximately 80%.

Preparation of tri-n-butyl(124)tin bromide

Approximately 1 g tetra-n-butyl(124)tin was suspended in 10 ml reagent grade anhydrous methanol. A stoichiometric amount of bromine dissolved in methanol was added dropwise to the organotin solution at room temperature and in dim light to reduce possible free radical reactions. Upon completion of the bromine addition, the solvent was removed by vacuum distillation. The crude product was cleaned by column chromatography using a 1.5 x 30 cm Florisil column. The reaction product mixture was eluted first with hexane to remove unreacted tetra-n-butyltin, then with 1:4 (v/v) ethyl acetate/hexane to recover the tri-n-butyltin bromide. Side products with two or more bromine groups remained on the column. The halide was produced in a 80% yield.

Derivatization with hexyl magnesium bromide

Butyltin halides were dissolved in hexane. An excess of 2.0 M hexylmagnesium bromide in diethylether was added and the samples were allowed to react for ~20 minutes at 50° C. The

samples were hydrolyzed with 1.0 N H_2SO_4, and the organic layer was recovered for GC/MS analysis.

RESULTS AND DISCUSSION

Tetraalkyltin compounds exhibit excellent chromatographic characteristics on non-polar columns. Compounds are not lost due to thermal degradation; the compounds are nonpolar, so better peak shape is attained. Simpler spectra, and hence greater sensitivity, is obtained because isotopic contribution from the halides is eliminated. Although trialkyltin halides chromatograph fairly well, the mono- and di-substituted alkyltins, which we wish to identify if present, do not. These require derivatization. Quantification without prior derivatization of alkyltin halides can also be a problem since anion exchange in the hot GC injector can occur. For identification purposes, however, we have included discussion of tri-n-butyltin bromide spectra.

For quantitative analysis, we routinely derivatize alkyltin halides with an excess of commercially obtained 2.0 M n-hexylmagnesium bromide in diethylether. Hexyl derivatives have advantages over alkyl derivatives that have shorter carbon chains. It is possible that butyltins could naturally degrade to tin-containing compounds with shortened alkyl chains. The hexyl derivatives cannot be confused with these possible degradation products, which might be the case if propyl or smaller groups were used to form the tetraalkyltin derivative. In addition, hexyl derivatives are less volatile, so their loss during concentration or clean-up procedures is reduced. Finally, the 28 amu mass separation between the resulting fragment ion clusters spreads out and clarifies the fragmentation pattern. The use of pentyl derivatives provides only a 14 amu mass separation which might cause some overlap between fragmenation patterns.

Tetraalkyltins exhibit mass spectra characterized by the successive loss of alkyl groups from the tin atom (16,17,18). The parent ion is weak or nonexistent. There is low abundance of tin-containing ions that have lost portions of the original alkyl chain. The most favorable ions are trisubstituted tin; monosubstituted tin atoms are also relatively abundant. Disubstituted tin ions are not very favorable.

For tetra-n-butyltin (Bu = butyl group), prepared from naturally occurring tin, the resulting ion fragments are shown in

Figure 1. Clusters of tin-containing ions center at m/z 121 (SnH^+), 179 ($BuSnH_2^+$), 235 (Bu_2SnH^+), and 291 (Bu_3Sn^+). The clusters are formed by the ten stable tin isotopes, their associated alkyl groups and one or more abstracted hydrogen atoms.

When naturally occurring tin is replaced with ^{124}Sn, the mass spectrum is greatly simplified. Major ions occur at m/z 124 (Sn^+), 125 (SnH^+), 127 (SnH_3^+), 181 ($BuSn^+$), 183 ($BuSnH_2^+$), 239 (Bu_2SnH^+) and 295 (Bu_3Sn^+) in Figure 2. The low intensity ions at m/z 184, 240 and 296 are ^{13}C-containing fragments.

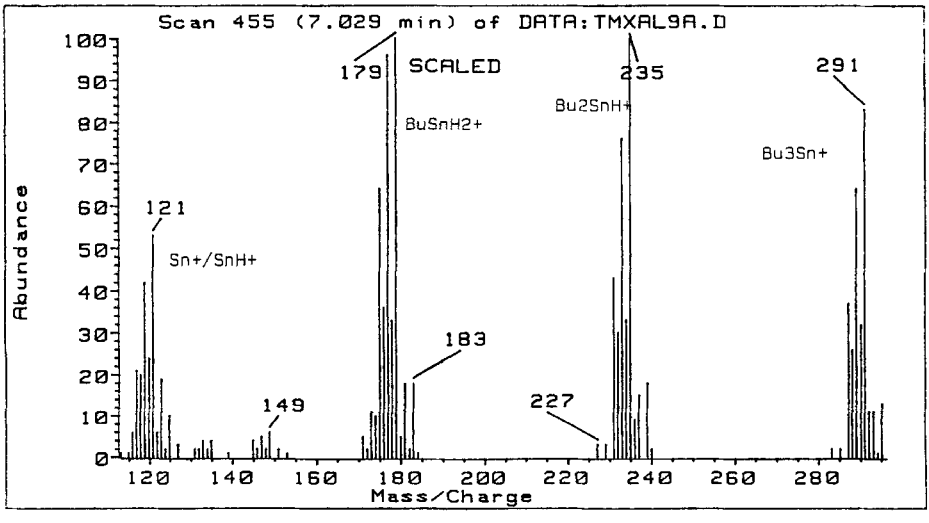

Figure 1 Mass spectrum of natural tetrabutyltin

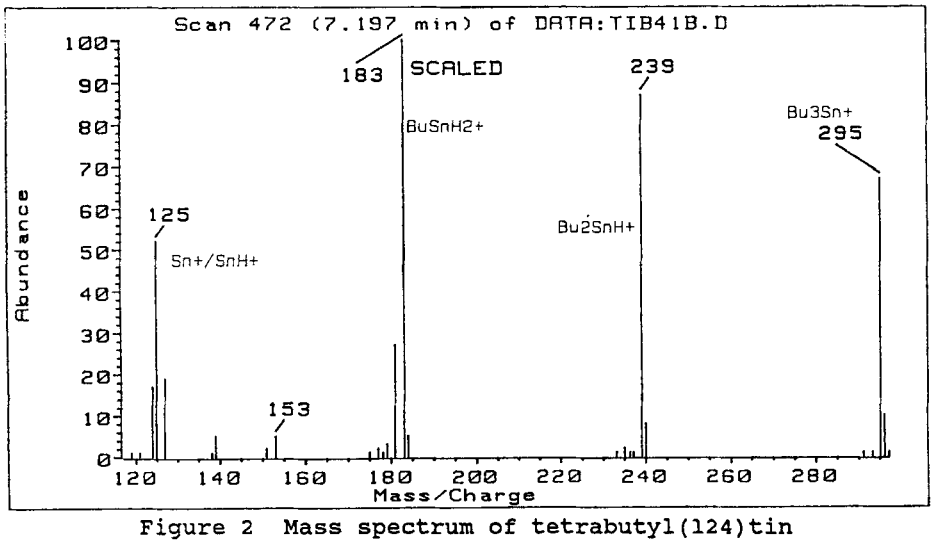

Figure 2 Mass spectrum of tetrabutyl(124)tin

For tri-n-butyl-n-hexyltin (Hx = hexyl) prepared from naturally occurring tin, the resulting fragment ions are shown in Figure 3. The clusters of tin-containing ions center at m/z 121 (SnH^+), 179 ($BuSnH_2^+$), 207 ($HxSnH_2^+$, 235 (Bu_2SnH^+), 263 ($BuHxSnH^+$), 291 (Bu_3Sn^+) and 319 (Bu_2HxSn^+). The ^{124}Sn-containing compound has the simplified spectrum shown in Figure 4. Major ions occur at m/z 124 (Sn^+), 125 (SnH^+), 127 (SnH_3^+), 181 ($BuSn^+$), 183 ($BuSnH_2^+$), 211 ($HxSnH_2^+$), 239 (Bu_2SnH^+), 267 ($BuHxSnH^+$), 295 (Bu_3Sn^+) and 323 (Bu_2HxSn^+).

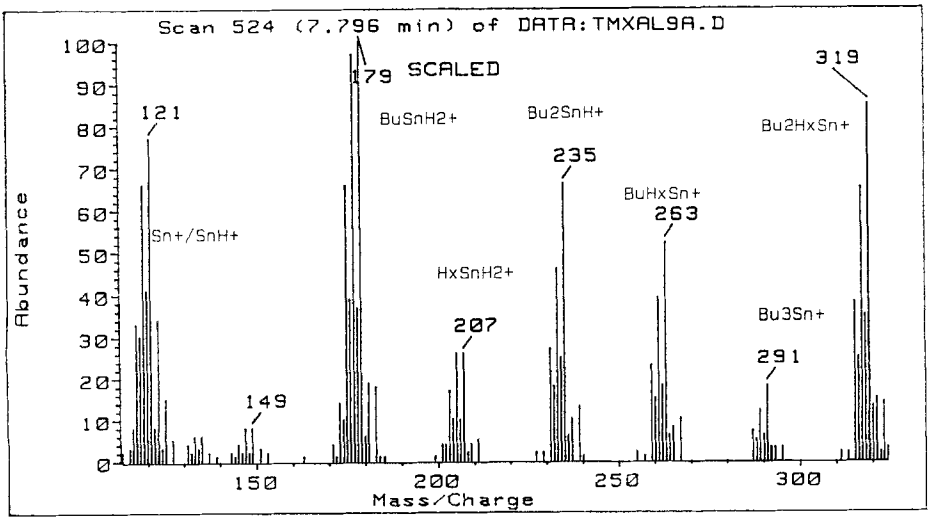

Figure 3 Mass spectrum of natural tributylhexyltin

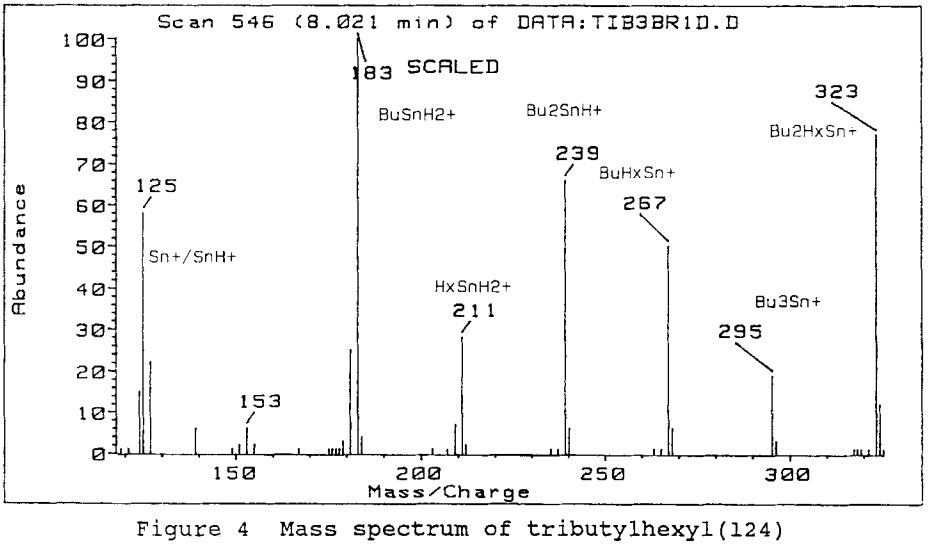

Figure 4 Mass spectrum of tributylhexyl(124)

Trialkyltin halides in general and tri-n-butyltin bromide in particular show a similar pattern of fragmentation to tetraalkyls, in that successive loss of the alkyl groups is favored over halide loss (19). As shown in figure 5, fragment ion clusters

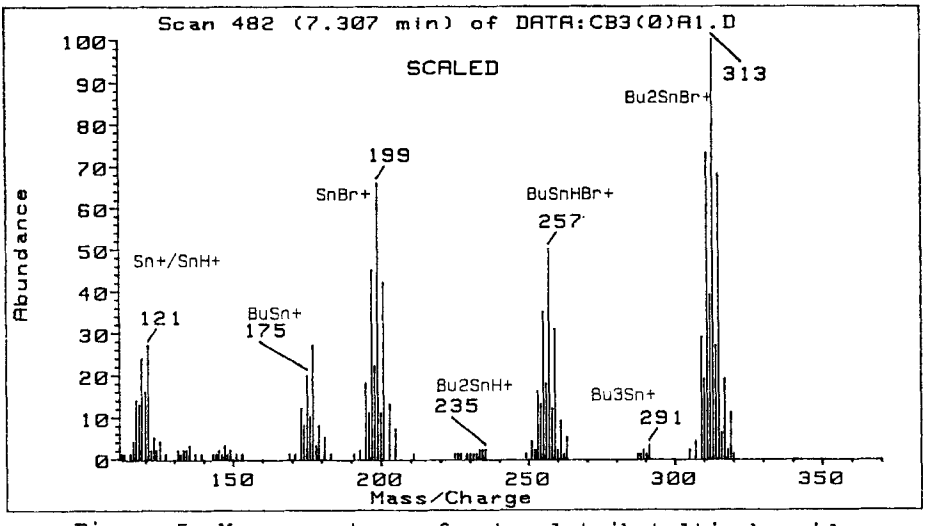

Figure 5 Mass spectrum of natural tributyltin bromide

occur at m/z 121, 175, 199, 257, and 313 for SnH^+, $BuSn^+$, $SnBr^+$, $BuSnHBr^+$, and Bu_2SnBr^+, respectively. The largest peak occuring between 100 and 350 m/z is Bu_2SnBr^+. The simplified spectrum for ^{124}Sn , in Figure 6, shows SnH^+ at m/z 125, $BuSn^+$ at m/z 181,

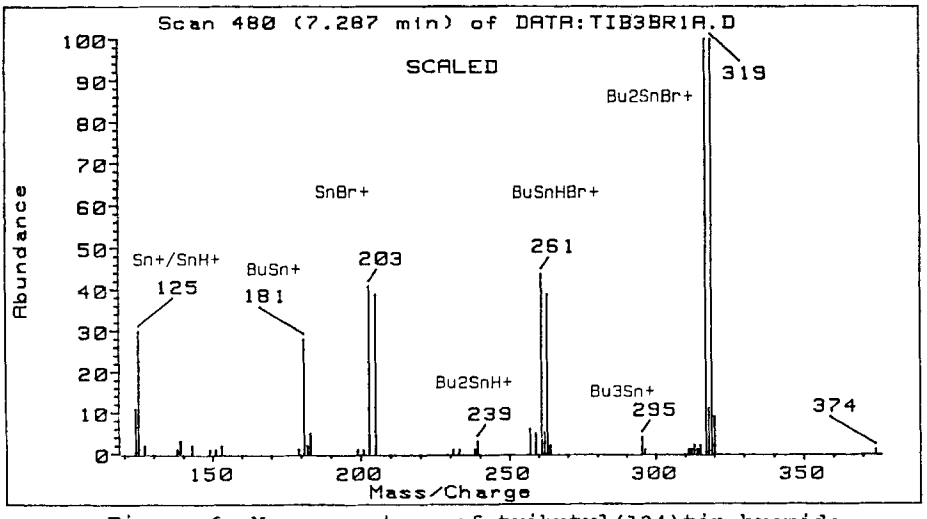

Figure 6 Mass spectrum of tributyl(124)tin bromide

$SnBr^+$ at m/z 203 and 205, $BuSnHBr^+$ at m/z 261 and 263, and Bu_2SnBr^+ at 317 and 319.

There are several advantages of using ^{124}Sn compounds as tracers. First, products resulting from chemical and biological processes can be readily identified and distinguished from naturally occurring tin compounds. For this reason, mass balance calculations are simplified and possible redistribution reactions may be resolved. Since these are stable isotopes, there is no additional hazard associated with their use.

The simplified spectra exhibited by these labelled compounds enhances the sensitivity of detection by mass spectrometry. In both full scan and selected ion monitoring, the tin-containing ions reaching the detector are limited to only a few discrete m/z values, rather than being dispersed over the ten stable tin isotopes in each cluster as in natural compounds. The most abundant natural isotope (^{120}Sn) is 32.4% of the total. In the labelled compound the ^{124}Sn is enriched to 97.4% of the total. Therefore, the theoretical improvement in sensitivity for a single isotope replacing the ten isotope cluster is about a factor of three . However, since we use several ions to insure positive identification of labelled and unlabelled butyltin compounds, the actual increase in sensitivity is only twofold because of computer limitations.

To determine detection limits we used the method of specified assurance probabilities (20,21). With our calibration design we could detect 1.1 micromolar tri-n-butyl-n-hexyltin with 95% confidence. With a similar similar design, we could detect 0.4 micromolar tri-n-butyl-n-hexyl(124)tin. With planned refinements in calibration design we should be able to reduce the detection limit for tri-n-butyl-n-hexyltin to about 0.2 micromolar and that for tri-n-butyl-n-hexyl(124)tin to about 0.1 micromolar. Where organotin compounds comprised of naturally occuring tin are present, a five per cent spike of labelled organotin must be added to perform a tracer experiment.

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REFERENCES

1. Drahowzal, F. A. and Wiesinger, F., Chem. Pham. Bull.23: 2944 (1975)

2. Klotzer, D., Isotopenpraxis 3: 128 (1976)

3. Meinema, H. A. and Martens, H. F., Synth. React. Inorg. Met.-Org. Chem. 7: 455 (1977)

4. Otto, P. Ph. H. L., Creemers, H. M. J. C. and Luijten, J. G. A., J. Labelled Cmpd. 2: 339 (1966)

5. Brown, R. A., Nazario, C. M., de Tirado, R. S., Castrillon, J. and Agard, E. T., Environ. Res. 13: 56 (1977)

6. Tenny, K. S. and Tenny, A. M. - J. Labelled Cmpd. 4: 54 1968)

7. Evans, W. H., Cardarelli, N. F. and Smith, D. J. - J. Toxicol. Environ. Health 5: 871 (1979)

8. Kimmel, E. C., Casida, J. E. and Fish, R. H. - J. Agric. Food Chem. 28: 117 (1980)

9. Bridges, J. W., Davies, D. S. and Williams, R. T. - Biochem. J. 105:1261 (1967)

10. Ward, G. S., Cramm, G. C., Parrish, P. R., Trachman, H. and Slesinger, A. - Aquatic Toxicology and Hazard Assessment: Fourth Conference, ASTM STP 737 (D. R. Branson and K.L. Dickson, Ed.) ASTM pp. 183-200 (1981)

11. Laughlin, R. B. Jr., French, W. and Guard, H. E. - Environ. Sci. Technol. 20: 884 (1986)

12. Lee, R. F. - Mar. Environ. Res. 17: 145 (1985)

13. Sheldon, A. W. and Slesinger, A. E. - Proc. Ann. Mar. Coatings Conf. 18: 18pp (1978)

14. Barug, D. and Vonk, J. W. - Pestic. Sci. 11: 77 (1980)

15. Stroganov, N. S., Parina, O. V. and Sorvachev, K. F. - Gidrobiol. Zhurn. 9(6): 59 (1973)

16. Occolitz, J. L. - Tetrahed. Lett. No. 5291 (1966)

17. Boue, S., Gielen, M. and Nasielski, J. - Bull. Soc. Chim. Belges 77: 43 (1968)

18. Chambers, D. B., Glockling, F. and Weston, M. - Chem. Soc. (A):1759 (1967)

19. Gielen, M. and Mayence, G. - J. Organomet. Chem. 12: 363 (1968)

20. Clayton, C. A., Hines, J. W. and Elkins, P. D. - Anal. Chem. 59: 2506 (1987)

21. Clayton, C. A. - "Calibration Designs for Detecting Presence or Absence of Analytes"; Research Triangle Institute Technical Report 3079/10-01F (1986)