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Use of mass spectroscopic techniques to elucidate the nature of the products of the oxidation of trimethylstibine in air†

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In view of the biological production of trimethylstibine ($(CH_3)_3Sb$) in the natural environment, the fate of this species when exposed to ambient oxygen has been studied. The results obtained show that the oxidation process leads to a complex series of products. Trimethylstibine oxide ($(CH_3)_3SbO$) and a range of cyclic and linear oligomers have been detected using positive ion electrospray and atmospheric pressure chemical ionization techniques and the mass spectroscopic (MS) features are discussed. Dimethyl antimony ($(CH_3)_2Sb$) species were not detected under the conditions used. The results from both MS techniques were similar. **Copyright © 2001 John Wiley & Sons, Ltd.**

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INTRODUCTION

Over the past 20 years there has been much evidence for the occurrence of methyl antimony species in the natural environment, but only recently has this evidence become more than tentative. Andreae and coworkers reported mono- and di-methyl antimony compounds in sea and river water.[1,2] Cullen and coworkers[3] and Craig *et al.*[4] have recently reported such species in freshwater biota and plants. The two latter groups have also demonstrated that $(CH_3)_3Sb$ can be produced above cultures of antimony(III) species with the fungus *Scopulariopsis brevicaulis*.[5–15] In one case, the aerobic phase required for active growth by the organism was replaced by an inert atmosphere (i.e. to reduce aerobic decomposition of $(CH_3)_3Sb$).[16] The antimony substrates used have included potassium antimony tartrate (PAT) and antimony(III) oxide. Challenger[17] suggested that biomethylation took place by way of a redox process involving oxidative addition to arsenic(III) (Fig. 1) with the methyl carbonium ion provided by *S*-adenosylmethionine (SAM). Though this method was proposed for arsenic, it is likely to be valid for the methylation of any species where the element has variable and achievable oxidation states separated by two units. It can be seen from Fig. 1 that mono-, di- and tri-methyl antimony species should, in principle, be capable of detection in the environment (assuming that end points exist corresponding to these stages in the Challenger mechanism). It is not possible to speculate at this point as to the existence of methyl antimony ribosides existing in the environment, akin to the arsenic examples. From Fig. 1 it can be seen that the $(CH_3)_3SbO$ is formed first and that $(CH_3)_3Sb$ is a reduction product. Nevertheless, we take it that the $(CH_3)_3Sb$ when produced and exposed to air is liable to oxidation.[18]

The analytical chemistry for methyl antimony species has been challenging. The volatile form ($(CH_3)_3Sb$) can be analysed directly, and the non-volatile mono- and di-methyl species can be converted to volatile CH_3SbH_2 and $(CH_3)_2SbH$ from the analyte by use of sodium tetrahydroborate ($NaBH_4$) and these can then be detected directly (care being taken to avoid any disproportionation).[4,5,9,11] However, the various arsenoribosides present in marine species, as well as arsenobetaine (AB) and arsenocholine (AC), cannot be deriva-

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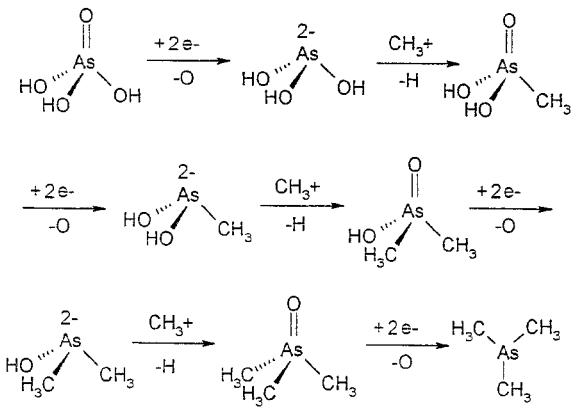

Figure 1 The Challenger arsenic biomethylation mechanism.

tized directly by this method. It cannot be assumed, therefore, that detection by hydride generation (to produce volatile species for gas chromatography–atomic absorption (GC–AA) detection, etc.) is possible for ALL methyl antimony species in the environment. Liquid chromatography–mass spectrometry (LC–MS) methods provide a powerful mechanism for the elucidation of species in solution, particularly where there is a major isotopic contribution that can aid the identification of the species observed. This is particularly helpful when it is noted that, in general, only pseudo-molecular ion data are available from LC–MS measurements.

It is important to characterize the fate of $(CH_3)_3Sb$ produced by biomethylation. It is likely to be toxic in itself, but also easily oxidized to $(CH_3)_3SbO$. It is then uncertain if the oxidized molecule (in whole or part) dimerizes or polymerizes to less-volatile species after initial oxidation to $(CH_3)_3SbO$.[18] From Fig. 1, as noted, it can be seen that $(CH_3)_3Sb$ may be produced directly in the methylation or it may be produced by reduction in the medium of final-stage $(CH_3)_3SbO$. In any case, $(CH_3)_3Sb$ has been detected in such experiments. (It is noted at this point that SbH_3 has not been reported following exposure of inorganic antimony compounds to micro-organisms despite a hypothesis to this effect.[19])

Parris and Brinckmann[18] made a study of the fate of $(CH_3)_3Sb$ in 1976. They concluded that most $(CH_3)_3Sb$ oxidizes rapidly to $(CH_3)_3SbO$ (identified by NMR). This is an important environmental and health conclusion, because, as the authors suggested, there would be little gaseous $(CH_3)_3Sb$ remaining to cause any toxicity problems in a manner analogues to Gosio gas, $(CH_3)_3Sb$. Nearly all the biomethylation product would be expected to be in a solid non-volatile state. Parris and Brinckmann[18] also observed small amounts of a dimethyl antimony product, which they identified by NMR as $(CH_3)_2Sb(OCH_3)$ — the latter methyl group coming from solvent methanol. Essentially, their main product on oxidation in air was suggested as $(CH_3)_3SbO$ with smaller amounts of dimethyl species, which they suggested existed polymerized as the species $[(CH_3)_2SbO(OH)]_n$. They also suggested the presence of insoluble antimony oxides resulting from $(CH_3)_3SbO$, but were unable to provide direct evidence.

Whilst this is an elegant analysis, the results were limited by the then lack of full speciation techniques, e.g. the separation and discrete identification of the principal components.

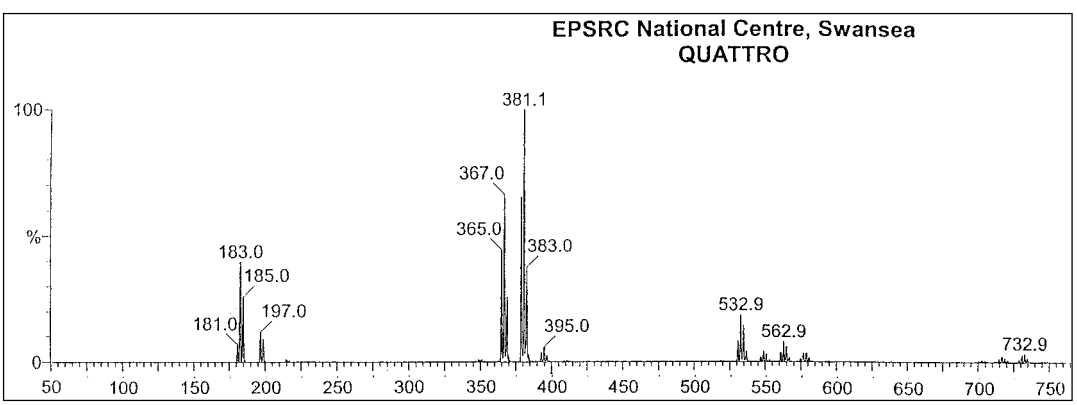

Figure 2 ESI mass spectrum of extract.

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In the present work, $(CH_3)_3Sb$ was allowed to oxidize in air and the resultant white solid was subjected to MS analysis. For MS study the solid could be extracted into water, methanol or aqueous methanol. The resultant soluble extracts were studied using positive ion electrospray (ESI) and atmospheric pressure chemical ionization (APCI) mass spectrometry methods. Results from methanol solutions were similar to those from aqueous solutions. A similar analysis of the oxidation products of the triphenyl antimony derivatives was carried out for comparison purposes. Use of ESI or APCI provides a separation (i.e. without chromatography) of the pseudo-molecular ions of non-volatile products, based on mass, therefore allowing a potentially more certain molecular identification than that available to Parris and Brinckmann,[18] who used NMR.

EXPERIMENTAL

Materials

Antimony(III) chloride (>99%) was obtained from Aldrich. Magnesium turnings were from Fischer Scientific. Methyl iodide (99% by GC) was from Aldrich. Iodine crystals, methanol (HPLC grade), acetonitrile (HPLC grade) and diethyl ether were from Fischer Scientific. Triphenylantimony oxide was obtained from Aeros, NJ, USA.

Sample preparation

Antimony(III) chloride ($SbCl_3$) was heated under vacuum (5.1×10^{-1} mbar) at 80 °C and sublimed out to a cold finger under nitrogen.

The Grignard reagent CH_3MgI was prepared from magnesium turnings (7.75 g, 0.32 mol) and CH_3I (30 g, 0.21 mol) in dry diethyl ether (200 cm^3) under a nitrogen atmosphere in a round-bottomed three-necked flask (500 cm^3). The CH_3I was added slowly over 30 min, sufficient to maintain reflux (water-cooled condenser). A crystal of iodine was added to promote the reaction. $SbCl_3$ was then added over 45 min (15 g, 0.066 mol in 200 cm^3 dry diethyl ether). After reaction (~1 h), the diethyl ether was removed under vacuum at 30 °C, and the product $(CH_3)_3Sb$ (b.p. 60 °C) was distilled off (still under N_2) at 78 °C at normal pressure. To study $(CH_3)_3SbO$ and its subsequent oxidation products the $(CH_3)_3Sb$ was oxidized by exposure to atmosphere. Then water, acetonitrile and methanol extracts of the oxidation products were subjected to mass spectrometric analysis. Only the soluble portion of the products of oxidation were subjected to MS analysis.

ESI and APCI mass spectral analyses

The dried oxidation product of $(CH_3)_3Sb$ was dissolved in water (100 mg in 5 cm^3 of water) and subjected to ESI and APCI mass spectrometry. Samples (20 μl) were injected directly into the MS interface using a methanol/water 20:80 mobile phase.

Electrospray analyses

Finnigan Mat 900XL and VG Quattro instruments were used in ESI positive ions mode. The cone voltage was 20 V. The mass range was 100 to >1000 with a resolution of ± 0.4 Da. The ESI work was carried out at the EPSRC National Mass Spectrometry Centre at Swansea, UK.

APCI analyses

A Hewlett Packard MSD instrument was used in APCI positive ions mode. The fragmentor voltage was 30 V. The mass range was 100–1500 with a resolution of ± 0.4 Da. The APCI analyses were carried out at De Montfort University, Leicester, UK.

RESULTS AND DISCUSSION

In both the ESI and APCI systems used in this investigation the species detected was the protonated ion, i.e. the $(M + H)^+$ species or the cationic molecular species and, for the former, the common ions therefore appear one mass unit higher than expected. In general, the results from all the MS investigations gave the same ionic species. In the APCI mass spectra the *m/z* 365 peaks were most abundant, whereas in the ESI spectra the group starting with *m/z* 379 was the most abundant. Other differences can be accounted for in terms of different relative abundances, which may reflect solutions of different concentrations being analysed.

Antimony has two isotopes, *m/z* 121 (57.2%) and 123 (42.7%), and the appropriate isotope pattern (two peaks equals one Sb atom; three peaks equals two Sb atoms, etc.) and relative abundances (RAs) can therefore be used to identify the number of antimony atoms in a particular ion. Throughout this

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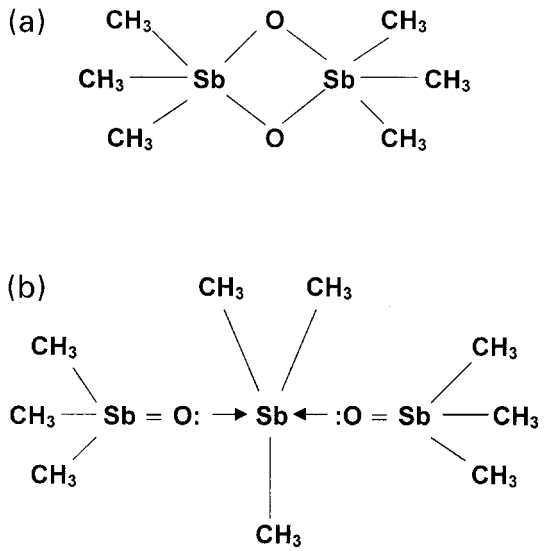

Figure 3 Structure of (a) $[(CH_3)_3SbO]_2$, the simplest of the structures proposed as cyclic species based on alternating Sb–O bonds and (b) structure number VI in Table 1.

work, the *m/z* values based on the lowest isotopic mass for any structure will be used for simplicity.

Triphenyl antimony oxide

The Ph_3Sb oxidized species (Ph_3SbO) was studied as it was thought it might provide a simple basis for comparison with methyl species. The molecular ion for this compound was observed at *m/z* 369 and 371 as the $(M + H)^+$ species, as expected for Ph_3SbO. The range of peaks observed in this area was: *m/z* 369 (RA 70%), 370 (RA 16%), 371 (RA 58%) and 372 (RA 14%). The RAs of the 369 and 371 peaks are, within experimental error, in the correct isotopic ratio for antimony, whereas the peaks at *m/z* 370 and 372 correspond to the ^{13}C analogues of 369 and 371 respectively. The relatively large contribution from the ^{13}C isotope (20% of the observed corresponding ^{12}C signal) results from the presence of 18 carbon atoms in the molecule, each contributing approximately 1.1% RA. There were no other antimony-containing species noted in the mass spectra, and thus there was no evidence for the presence of species containing two or more antimony atoms.

Trimethyl antimony species

In the current investigation all the extracts of the oxidized $(CH_3)_3Sb$ gave similar MS results, the clearest data being obtained from the methanol solution as a result of greater solubility. In the ESI data (Fig. 2) a series of peaks can be seen corresponding to compounds containing one (*m/z* 181–199), two (*m/z* 365–383), three (*m/z* 531–597) and four (*m/z* 699–753) antimony atoms. The proposed structures for the ten most abundant of these compounds, based on the mass spectral data, are shown in Table 1. For species containing two or more antimony atoms, the structures are proposed either as cyclic species based on alternating Sb—O bonds (structures IV, VII and X) or as Lewis acid–Lewis base complexes (structures VI, VIII and IX). The simplest of the former, $[(CH_3)_3SbO]_2$, is shown in Fig. 3.

Our discussions are based on the species observed in greatest abundance, i.e. those observed at *m/z* 181, 197, 365, 379, 531, 547, 561, 713 and 729 (the lowest *m/z* value for each group is cited). The *m/z* data cited in Table 1 correspond mainly to

Table 1 Proposed structures from the most abundant ions observed in the mass spectral analyses

Observed m/z	Proposed neutral structure	Structure number
181/3	$(CH_3)_4Sb^{+a}$	I
183/5	$(CH_3)_3SbO$	II
197/9	$(CH_3)_4SbO^{+a}$	III
365/7/9	$[(CH_3)_3SbO]_2$	IV
379/81/3	$[(CH_3)_4Sb]_2O$	V
531/3/5/7	$[(CH_3)_3SbO]_2(CH_3)_3Sb^b$	VI
547/9/51/3	$[(CH_3)_3SbO]_3$	VII
561/3/5/7	$[(CH_3)_4SbO]_2(CH_3)_3Sb^b$	VIII
713/5/7/9/1	$[(CH_3)_3SbO]_3(CH_3)_3Sb^b$	IX
729/31/3/5/7	$[(CH_3)_3SbO]_4$	X

[a] Possible fragment ion observed as such, no neutral species.
[b] Lewis acid–Lewis base structures.

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the protonated molecular ions, and hence the ions at *m/z* 183 and 185 are protonated $(CH_3)_3SbO$ (i.e. $(CH_3)_3SbOH^+$), whereas the peak at *m/z* 181 and a contribution to 183 are thought to be $(CH_3)_4Sb^+$. The protonated dimer of $(CH_3)_3SbO$, i.e. $[(CH_3)_3SbO]_2H^+$ (structure IV), is observed at *m/z* 365/7/9. The ions at *m/z* 379, 381 and 383 are consistent with the loss of one oxygen atom from $[(CH_3)_3SbO]_2H^+$ with the subsequent inclusion of two methyl groups (structure V) or oxidations of $(CH_3)_4Sb^+$. The ions at 197 and 199 (structure III) are assigned as fragment ions produced in the mass spectrometer, since their relative abundance increases with ion energy. These would most readily be derived from $[(CH_3)_4Sb]_2OH^+$ (structure V). This fragmentation phenomenon has recently been reported for similar antimony-containing compounds by Furuta *et al.*[20]

Combination of three $(CH_3)_3SbO$ molecules will produce the species $[(CH_3)_3SbO]_3H^+$ with ions at *m/e* 547, 549, 551 and 553 (structure VII), and the combination of two $(CH_3)_3SbO$ molecules and one $(CH_3)_3Sb$ will produce the protonated Lewis acid–Lewis base structure VI seen at *m/e* 531, 533, 535 and 537. Structure VIII (also a Lewis acid–Lewis base complex) can be envisaged as being derived from the loss of one oxygen atom from structure VII, in a manner analogous to compound IV, which is then replaced by two methyl groups. The groups of ions between *m/z* 699 and 751 (see Fig. 2), in general, show five major peaks per group, indicative of species containing four antimony atoms. However, the group of peaks between *m/z* 713 and 723 consists of six isotopic peaks, suggesting the presence of either five antimony atoms per compound or the presence of two compounds with overlapping peaks. The Lewis acid–Lewis base structure (IX) could account for one of these species with masses 713/5/7/9/21. An anticipated cyclic compound consisting of four $(CH_3)_3SbO$ molecules was noted at *m/z* 729 (structure X). In repeated experiments, minor differences in the relative abundances of the compounds containing four antimony atoms were detected. These variations probably result from the overall low level of the signal at these masses.

DISCUSSION AND CONCLUSION

From the results obtained there appears to be no significant difference in the nature of the results obtained as a function of any solvents present. There is no evidence, from the mass spectral data, for the incorporation of hydroxide ions into any of the compounds proposed. This is perhaps not surprising, since Furuta *at al.*[20] reported that the major ions in the mass spectrum of $(CH_3)_3Sb(OH)_2$ occurred at *m/z* 183 and 365, i.e. the protonated oxide and dimer respectively, as noted here. There authors did not report on oligomeric species.

There was no evidence, from the major ions observed, for the presence of antimony plus CH_3O (*m/e* 31) species, as suggested by Parris and Brinckmann from the NMR spectra. The presence of many low-intensity ions in the mass spectral data (Fig. 2) could conceivably correspond to such species. Furuta *et al.*[20] suggested that such species could result from the fragmentation of the dimers in the mass spectrometer, but this source of these ions would not explain the NMR observations of Parris and Brinckmann.[18]

In this investigation there is no evidence for the presence of solvated ions, e.g. as reported by Lintschinger *et al.*[21] in the analysis of $(CH_3)_3SbCl_2$ in solution in a mixture of H_2O:methanol:acetic acid. The main peaks observed by Lintschinger *et al.*[21] corresponded to protonated or solvated (H_2O or CH_3OH) versions of $(CH_3)_3SbOCl$, and were $(CH_3)_3Sb(OH)^+$ (*m/z* 183, 185), $(CH_3)_3SbO(H_2O)H^+$ (*m/z* 201, 203) and $(CH_3)_3SbOCl(MeOH)^+$(*m/z* 249, 251, 253). Lintschinger *et al.*[21] did not report the formation of any cyclic species. There is also precedent for $(CH_3)_4Sb^+$ by way of the known $(CH_3)_4As^+$.[22]

It appears, therefore, that $(CH_3)_3Sb$ does not oxidize as rapidly or as completely as estimated by Parris and Brinckmann,[18] particularly in the gas phase. These authors estimated rate constants for the oxidations as follows:

	K (M^{-1} s^{-1}) gas phase	methanol phase
$(CH_3)_3As$	$<10^{-2}$	10^{-6}
$(CH_3)_3Sb$	$>10^{-2}$	10^{3}

However, the observation of non-oxidized $(CH_3)_3Sb$ produced from culture media,[5,9] which are not entirely anaerobic, suggests that $(CH_3)_3Sb$ has reasonable stability. Only a small proportion of the inorganic antimony present in the culture media is converted to methyl forms (the proportion of methyl to inorganic species in biota is around 0.5%; however, this would not allow for any $(CH_3)_3Sb$ lost to atmosphere[4]). Clearly, some of the $(CH_3)_3Sb$

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produced in an aqueous culture medium during an aerobic phase (e.g. using *S. brevisaulis*) is potentially capable of conversion to $(CH_3)_3SbO$ and other species. That hydride generation of antimony-containing culture media[23] produces $(CH_3)_3Sb$ demonstrates that forms such as those discussed above are present. (In analogy with the arsenic case, it is assumed that any antimony analogues of arsenobetaine, arsenocholine or the methyl arsenioribosides might not produce $(CH_3)_3Sb$ on hydride generation. This is an important assumption requiring to be tested.) It is noted that Andrewes *et al.*[23] and Jenkins *et al.*[16] also detected $(CH_3)_2SbH$ on hydride generation but not CH_3SbH_2 from biota samples.[16]

The present work shows that if $(CH_3)_3Sb$ is allowed to oxidize, species containing one to five $(CH_3)_3SbO$ molecules chemically bound together are produced in aqueous or culture media.

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REFERENCES

1. Andreae MO, Asmode JF, Foster P, Van't Dack L. *Anal. Chem.* 1981; **53**: 1766.
2. Andreae MO, Froelich Jr. PN. *Tellus* 1984; **366**: 101.
3. Dodd M, Rergantis SA, Cullen WR, Li H, Eigendorf GK, Reimer KJ. *Analyst* 1996; **121**: 223.
4. Craig PJ, Forster SN, Jenkins RO, Miller DP. *Analyst* 1999; **124**: 1243.
5. Feldmann J, Koch I, Cullen WR. *Analyst* 1998; **123**: 815.
6. Hirner AV, Feldmann J, Krupp E, Grumping R, Gozud R, Cullen WR. *Org. Geochem.* 1998; **29**: 1765.
7. Andrewes P, Cullen WR, Polishchuk E. *Appl. Organomet. Chem.* 1999; **13**: 659.
8. Cullen WR, Li H, Hewitt G, Reimer KJ, Zolunardo N. *Appl. Organomet. Chem.* 1994; **8**: 303.
9. Cullen WR, Li H, Rergantis SA, Eigendorf GK, Mosi AA. *Appl. Organomet. Chem.* 1994; **9**: 507.
10. Gurleyuk H, Van Fleet Stalder V, Chasteen TG. *Appl. Organomet. Chem.* 1997; **11**: 471.
11. Jenkins RO, Craig PJ, Goessler W, Miller DP, Ostah N, Irgolic KJ, *Environ. Sci. Technol.* 1998; **32**: 882.
12. Craig PJ, Jenkins RO, Dewick R, Miller DP. *Sci. Total. Environ.* 1999; **229**: 83.
13. Jenkins RO, Craig PJ, Miller DP, Stoop LCAM, Ostah N, Morris T-A. *Appl. Organomet. Chem.* 1998; **12**: 449.
14. Jenkins RO, Craig PJ, Goessler W, Irgolic KJ. *Human Exp. Toxicol.* 1998; **17**: 231.
15. Jenkins RO, Craig PJ, Goessler W, Irgolic KJ. *Human Exp. Toxicol.* 1998; **17**: 138.
16. Jenkins RO, Morris T-A, Craig PJ, Ritchie AW, Ostah N. *Sci. Total Environ.* 2000; **250**: 73.
17. Challenger F. *Chem. Rev.* 1945; **36**: 315.
18. Parris GE, Brinkman FE. *Environ. Sci. Technol.* 1976; **10**: 1128.
19. Richardson BA. *Lancet* 1995; **395**: 1045.
20. Furuta N, Takeda A, Zheng J. *J. Anal. At. Spectrom.* 2001; **16**: 62.
21. Lintschinger J, Schramel O, Keltrup A. *Fresenius. J. Anal. Chem.* 1998; **361**: 96.
22. Shiomi K, Horiguchi Y, Kaise T. *Appl. Organomet. Chem.* 1988; **2**: 385.
23. Andrewes P, Cullen WR, Feldmann J, Koch I, Polishchuk E, Rimer KJ. *Appl. Organomet. Chem.* 1998; **12**: 827.

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