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Ion chromatographic analysis of the purity and synthesis of sulfonium and selenonium ions

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ABSTRACT

The use of single-column ion chromatography with conductometric detection was shown to be useful for the analysis of sulfonium and selenonium ions. A Hamilton PRP X-200 cation column was eluted with either solvent A (5 m*M* nitric acid in 30% methanol) or solvent B (4 m*M* nitric acid). With solvent B, trimethylsulfonium ion was separated from trimethylselenonium ion. With solvent A, amounts of trimethylsulfonium ion from 2 to 250 nmol were detected with a linear response. The retention times and response factors for a series of sulfonium ions with various organic groups were determined. In general the ions with more hydrophobic groups eluted later, but all had similar response factors. The method was shown to be useful for optimizing conditions for the synthesis of methylsulfonium ions, specifically the reaction of methyl iodide with diallyl sulfide.

INTRODUCTION

In the course of studies on the enzyme thioether methyltransferase which methylates thioethers or selenoethers to form the corresponding methylonium ions [1] it was necessary for this laboratory to chemically synthesize and characterize various sulfonium ions for use as chromatographic standards [2]. It became apparent that a facile method was needed to monitor the purity of these compounds, particularly in view of the multiple products possible from the reaction of methyl iodide with a thioether [3]. This problem is illustrated specifically in Fig. 1 which shows that the nominal synthesis of DAMS (see Table I for abbreviations) from diallyl sulfide and methyl iodide can result in three additional sulfonium ions by various cycles of dealkylation by I^- and subsequent realkylation. A similar outcome may result starting with other thioethers.

In previous studies the sulfonium and selenonium ions of interest were radioactively labeled either chemically or metabolically, separated by conventional cation-exchange high-performance liquid chromatography (HPLC), and detected by liquid scintillation counting of collected fractions [1,2]. However, a method of analysis not requiring radioactive detection was desired for two reasons. First, during the chemical synthesis of methylsulfonium ions using [^{14}C]methyl iodide a non-radioactive product, exemplified by TAS in Fig. 1, can be

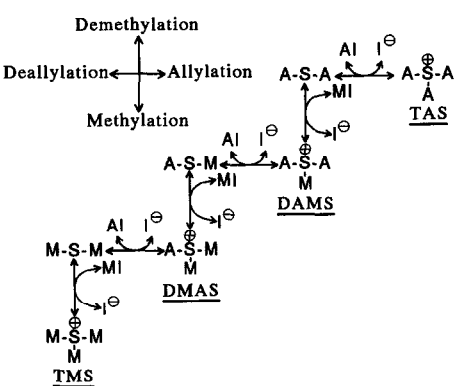

Fig. 1. Potential sulfonium ion products starting with diallyl sulfide and methyl iodide. M = Methyl group; A = allyl group. Abbreviations of sulfonium ions are underlined.

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obtained and would not be detected by the above method. Second, further studies of synthesis and excretion of methylsulfonium ions in intact animals would be facilitated by a direct detection method, and would be required in the case of human studies since the use of radioactivity would be precluded.

Most of the sulfonium and selenonium compounds of interest lack chromophores or fluorophores which prevents their detection by flow monitors sensitive to such groups. The ion chromatographic fractionation of triethylsulfonium and TMS and their detection by conductivity with the use of a suppressor column have been reported [4]. Therefore we investigated the analysis of sulfonium and selenonium ions with the simpler system of column ion chromatography with conductometric detection. This paper describes the use of the simplest trialkylsulfonium ion, TMS, to determine the sensitivity and dynamic range of single-column ion chromatography for analysis of this class of compounds. The effects on retention time of replacing the sulfur of TMS with selenium, and of varying the structures of the organic groups of sulfonium ions, are demonstrated. Finally the utility of this method of analysis in monitoring the temporal appearance of sulfonium ions produced by varying the ratio of methyl iodide/thioether is illustrated using diallyl sulfide.

EXPERIMENTAL

Apparatus

The HPLC system consisted of a Wescan Model 315 conductivity detector and Spectra-Physics 8800 pump, 8780 autosampler and 4290 integrator. System control and data capture were provided by Spectra-Physics Chromstation/2 software run with an IBM PS/2 Model 50 computer. The column used was a 15 × 0.46 cm I.D. Hamilton PRP X-200 cation column obtained from Rainin Instrument (Woburn, MA, USA).

Reagents

Methyl iodide, TMS iodide, thioethers and dimethyl selenide were obtained from Aldrich (Milwaukee, WI, USA). All other chemicals were generally of the highest grade obtainable.

Onium ion synthesis and characterization

The synthesis and characterization by low-resolution fast atom bombardment mass spectrometry (FAB-MS) has been described for all the sulfonium ions used here [2] except those containing allyl groups. The latter were prepared in 5-ml batches under conditions yielding a mixture of TAS, DAMS and DMAS (19 h, equimolar diallyl sulfide and methyl iodide; see Results). When this mixture was extracted into water as below for TMSe and then subjected to low-resolution FAB-MS, it showed m/z of 155, 129 and 103 corresponding, respectively, to TAS, DAMS and DMAS in the proportions found in the mixture by ion chromatography. TMSe was synthesized by reaction for 24 h in a total volume of 5 ml containing 1 *M* methyl iodide and 1 *M* dimethyl selenide in methanol. At the end of this time 5 ml each of water and diethyl ether were added, and the suspension was shaken and allowed to settle in a separatory funnel. The resulting aqueous phase was similarly extracted twice more with 5 ml of ether, and the final aqueous phase containing TMSe iodide was lyophilized to dryness. This product was used without further characterization.

Time course of the reaction of diallyl sulfide and methyl iodide

Reaction mixtures with total volumes of 1 ml in methanol contained 0.5 *M* diallyl sulfide and either 0.5 or 2.5 *M* methyl iodide at room temperature. Sampling was accomplished by placing reaction mixtures in vials in the autosampler with the HPLC system programmed to inject a sample of 3 μl from each vial at hourly intervals over a 10-h period. Additional samples were run at 18–19 h. Solvent A was used to elute all samples.

Chromatography

Solvent A contained 5 m*M* nitric acid in methanol–water (30:70) and was used for all analyses other than the separation of TMS and TMSe shown in Fig. 2, which utilized solvent B consisting of 4 m*M* nitric acid in water. Both solvents were run at a flow-rate of 2 ml/min. Injection volumes varied depending on the sample but were in the range from 3 to 100 μl. The detector range was set at 0.1 μS per 10 mV with the integrator attenuation set from 32 to 256 mV full scale depending on the anticipated peak sizes. Before use each day the column was regener-

ated by injection of 100 μl of 4 *M* nitric acid and equilibrated with the appropriate solvent.

RESULTS AND DISCUSSION

The initial experiments utilized commercially available TMS and chemically synthesized TMSe to establish the applicability of ion chromatography in the analysis of this type of compound. Typical elution profiles using solvent B for TMS, TMSe and a mixture of the two are shown in Fig. 2. The ability to separate these two closely related ions encouraged further exploration of the sensitivity and dynamic range of the method for TMS.

Amounts of TMS ranging from 2 to 250 nmol were next analyzed using solvent A, and the results are shown in Fig. 3. Panel A shows the integrator peak area *versus* amount of TMS. Linearity was observed over the entire range tested with a response factor (slope) of 42 300 μV · s/nmol. Amounts of TMS greater than 250 nmol exceeded the capacity of the detector at the 0.1 μS per 10 mV range. Less sensitive (higher capacity) settings are available, but some peak distortion was evident at sample loads greater than 250 nmol. The data at lower TMS levels are shown in the inset, which reveals the limit of detection to be 2 nmol. Thus at a single-detector range setting the dynamic range is greater than 100-fold. More sensitive detector range settings of 0.05 and 0.01 μS per 10 mV were available, but much higher noise levels prevented their use. The 2-nmol sensitivity may be sufficient for further biochemical studies of thioether methylation since this would correspond to 100 μl of a 20 μM solution of sulfonium ion. Such concentrations might be expected in urine of animals treated with doses of thioethers up to 1 mmol/kg. However, a sensitivity of 2 nmol would not be sufficient to detect the quantities of TMSe excreted in urine by rats or humans under conditions of normal selenium intake without extensive concentration by sample processing [5].

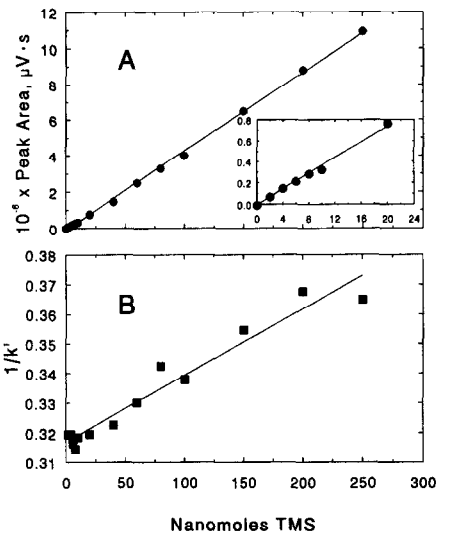

Fig. 3. Sensitivity, range and capacity factor for ion chromatography of TMS. Amounts of TMS from 2 to 250 nmol were eluted with solvent A. Panel A shows the linear range and detection limit for TMS. The inset is an expanded view of the lower end of the sample range. Panel B shows the linear dependence of the inverse of the capacity factor (k') on sample amount.

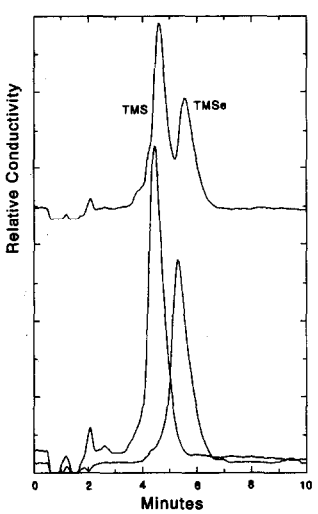

Fig. 2. Ion chromatographic separation of TMS and TMSe. Individual samples of 50 nmol of TMS and 40 nmol of TMSe (lower curves) and a mixture of 25 nmol of TMS and 20 nmol of TMSe (upper curve) were eluted with solvent B.

Fig. 3B shows that the inverse of the capacity factor (k') for TMS on the PRP X-200 column varies linearly with the sample load, which is predicted by the dual-retention model of ion chromatography [6]. In more practical terms the retention time of TMS varied from 4.17 min at 2 nmol to 3.74 min at 250 nmol. With biological samples having high total cation content, it may be difficult to identify peaks by comparison of retention times to those of exter-

TABLE I

RETENTION TIMES AND RESPONSE FACTORS OBTAINED DURING ION CHROMATOGRAPHY OF SULFONIUM AND SELENONIUM IONS

Cation[a]	Retention time[b] (min)	Response factor[b] (μV · s/nmol)
Potassium	3.54	36 800
Trimethyl (TMS)	3.97 (4.54)	42 300
Dimethyl-2-hydroxyethyl (DMHES)	4.00	50 500
Trimethylselenonium (TMSe)	4.25 (5.67)	48 400
2-Hydroxyethylethylmethyl (HEEMS)	4.34	42 300
Diethylmethyl (DEMS)	4.74	43 300
Tetramethylenemethyl (TMMS)	4.76	42 700
Dimethylpropyl (DMPS)	5.38	50 100
Dimethylallyl (DMAS)	5.50	N.D.
2-Chloroethylethylmethyl (CEEMS)	7.68	41 600
Diallylmethyl (DAMS)	7.80	N.D.
Triallyl (TAS)	11.52	N.D.

[a] Abbreviations in parentheses are used in the text. All cations other than K^+ and TMSe are sulfonium ions.

[b] Data given were obtained with solvent A, except for those retention times in parentheses which were obtained with solvent B. Samples contained no more than 50 nmol of each ion. N.D. means not determined.

nal standards. Duplicate runs with one sample containing an added internal standard would provide more definitive peak identification.

The next feature examined was the dependence of retention time on the structure of the onium ion, and the results are shown in Table I arranged in order of increasing retention time. An inorganic cation, K^+, was included for comparison. Samples contained no more than 50 nmol to minimize load effects on retention times. All of the organic cations eluted later than K^+, apparently due to hydrophobic interaction with the poly(styrene–divinylbenzene) resin. This interaction necessitated the inclusion of the organic modifier in solvent A (5 m*M* nitric acid in 30% methanol), since the sulfonium ions with higher carbon content eluted late and in broad peaks in solvent B (4 m*M* nitric acid). TMSe eluted later than TMS in both solvents consistent with the greater electronegativity of the selenium atom imparting more basicity to its onium ion.

Comparison of the retention times of the various sulfonium ions gives greater insight into the separation mechanism. In general, higher carbon content led to stronger retention. An interesting comparison is between DEMS and DMPS which have identical compositions, $(CH_3)_3(CH_2)_2S$; however, DMPS with the longest hydrocarbon chain eluted later. Another 5-carbon sulfonium ion, TMMS (S-methyltetrahydrothiophene), of slightly lower hydrogen content, $CH_3(CH_2)_4S$, had essentially the same retention time as DEMS. Substitution with a polar group such as hydroxyl decreased retention (DEMS *versus* HEEMS), while halide substitution greatly increased retention (DEMS *versus* CEEMS). Finally, unsaturation slightly increased retention (DMPS *versus* DMAS).

All of the organic ions gave response factors similar to each other and not greatly different from that of K^+. The most reliable response factors in Table I are those for TMS, TMSe and TMMS which were obtained as dry solids. All the other sulfonium ions gave oils or pastes upon drying which made accurate weighing difficult. Response factors were not obtained for the allylsulfonium ions, DMAS, DAMS and TAS, because they tended to decompose and rearrange when concentrated as halide salts. However, it appears safe to assume that they, like most other sulfonium and selenonium ions, would have response factors of approximately 45 000 μV s/nmol in this HPLC system.

The synthesis of methylsulfonium ions by reaction of thioethers and methyl iodide can lead to

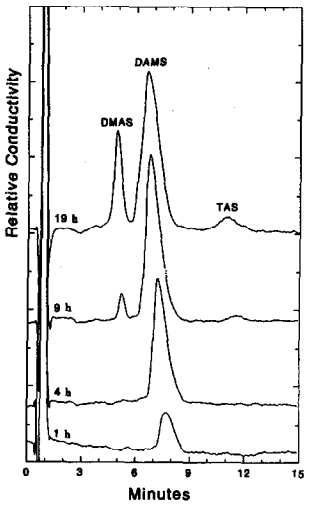

Fig. 4. Representative elution profiles of samples taken during the reaction of methyl iodide and diallyl sulfide. Samples of 3 μl from reaction mixtures containing 0.5 *M* each of methyl iodide and diallyl sulfide were run at the times indicated using solvent A.

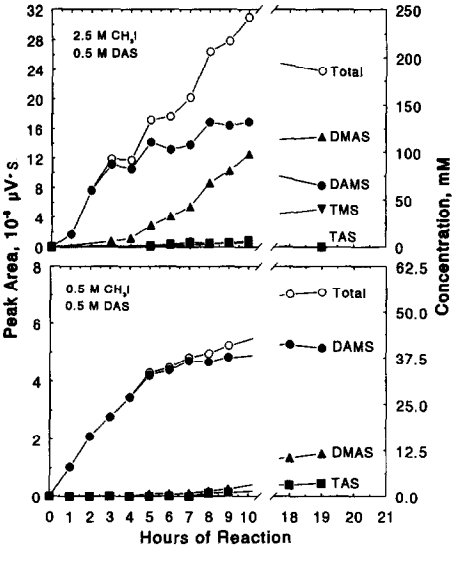

Fig. 5. Rates of appearance of sulfonium ions during the reaction of methyl iodide and diallyl sulfide. Samples of 3 μl were analyzed at the times indicated as in Fig. 4. The ordinates on the left are the integrated peak areas for sulfonium ions in the 3-μl samples. The concentration scales on the ordinates on the right assume a response factor of 44 000 μV · s/nmol for sulfonium ions. The concentrations of the two reactants are shown on each panel with diallyl sulfide abbreviated as DAS.

several undesired products as illustrated in Fig. 1. Ion chromatography proved to be very useful in obtaining optimal yield and purity. Representative elution profiles of samples obtained at various times during the reaction of 0.5 *M* each of methyl iodide and diallyl sulfide are shown in Fig. 4. For the first 4 h, only the desired product DAMS was evident. By 9 h DMAS and TAS appeared and were major contaminants after 19 h. A more quantitative summary of these data is shown in the lower panel of Fig. 5. At this equimolar ratio of methyl iodide and diallyl sulfide, DAMS was the major product after 19 h, and no TMS was synthesized. At 4 h, when DAMS was the only sulfonium product, its concentration of 27 m*M* corresponded to 5.4% conversion of the 500 m*M* diallyl sulfide. Increasing the ratio of methyl iodide/diallyl sulfide to 5:1 gave a more rapid initial rate of DAMS synthesis, as shown in the top panel, but extended reaction time led to the major product being DMAS with heavy contamination by TMS. Because of the high total methyl group concentration only a trace of TAS was detectable midway through the reaction period which disappeared by 19 h. At 3 h, when DAMS was the only sulfonium product, its concentration of 90 m*M* corresponded to 18% conversion of the 500 m*M* diallyl sulfide. Thus one could conclude that use of the higher methyl iodide concentration for 3 h would give the highest yield of pure DAMS. An additional advantage of this method is that the ion chromatographic analysis takes a relatively short time compared to the rate of methylation, permitting reactions to be stopped when the first trace of undesired product appears. More thorough studies may reveal conditions giving even higher yields of DAMS, but the above results establish the utility of ion chromatography in monitoring sulfonium ion synthesis.

In summary, ion chromatography with conductometric detection is shown to be a facile and sensitive method for the detection of sulfonium and selenonium ions. The technique should be useful in further studies of the chemistry and biochemistry of these compounds.

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