ISOLATION OF DIMETHYL DISELENIDE AND OTHER VOLATILE SELENIUM COMPOUNDS FROM ASTRAGALUS RACEMOSUS (PURSH.)

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Summary

Volatile selenium compounds from intact Astragalus racemosus plants and from oven-drying tops or roots of the same species were collected on activated charcoal and fractionated according to solubility in water or diethyl ether.

The ether-soluble fraction contained two compounds which could be separated by gas–liquid chromatography. One of these compounds was shown to be dimethyl diselenide. The other compound has not been positively identified.

The water-soluble fraction contained two as yet unidentified selenium compounds separable by anion-exchange column chromatography. The compounds appeared to be similar to, or identical with, the compounds isolated from lucerne in a previous study.

Intact plants released the same four volatile selenium compounds as oven-drying tops or roots, but the yield of these compounds from oven-drying tops or roots was much greater than from intact plants.

I. INTRODUCTION

It has been suggested that the distinctive, unpleasant odour of Astragalus plants containing high concentrations of selenium is due to the release of a volatile selenium compound, possibly dimethyl selenide (Rosenfeld and Beath 1964; Virupaksha and Shrift 1965). Recently Lewis, Johnson, and Delwiche (1966) confirmed the release of selenium in volatile form from the tops of intact plants of A. racemosus (Pursh.) and showed that the selenium released could be trapped very effectively on activated charcoal. However, the identity of the volatile selenium compound or compounds released was not established.

Research on the odour and flavour of onions and other edible plant species has shown that higher plants are capable of producing a wide variety of volatile sulphur compounds, including mercaptans, sulphoxides, and alkyl mono-, di-, and tri-sulphides (Ballance 1961; Carson and Wong 1961; Gumbmann and Burr 1964; Oaks, Hartman, and Dimick 1964). Since selenium is known to substitute for sulphur in a number of biological processes, it was reasoned that the characteristic odour of seleniferous vegetation might result from the substitution of selenium for sulphur in one or more of these volatile sulphur compounds. The characterization of selenium analogues of some of these compounds by gas–liquid chromatography was described in an earlier publication (Evans and Johnson 1966).

This paper reports results from an experiment in which four volatile selenium compounds from A. racemosus were isolated, and one of them subsequently identified by gas chromatography.

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II. Methods

(a) Plant Culture

Seeds of *A. racemosus* were soaked for 20 min in concentrated sulphuric acid (Trelease and Trelease 1939), rinsed for 4 hr in distilled water, and then spread out to germinate on moist cheesecloth. After 8 days, selected seedlings were transferred to 4-litre beakers of aerated Hoagland solution (Hoagland and Arnon 1950). High- and low-selenium treatments were imposed on duplicate sets of cultures. In high-selenium cultures, sodium selenite was added to give a selenium concentration of 300 μg-atoms/l. No stable selenium was added to low-selenium cultures. Both sets received H$_2$SeO$_3$ of high specific activity (29·1 c/g Se) at a rate of 25 μc/l.

(b) Collection and Characterization of Volatile Selenium Compounds

Volatile compounds from intact plants were collected on active carbon as described by Lewis, Johnson, and Delwiche (1966), and from tops or roots during oven-drying, as described by Asher, Evans, and Johnson (1967). Volatiles were extracted from the carbon with various solvents including acetone, carbon disulphide, ethanol, water, and cyclohexane. In each case 0·5 g carbon was extracted with 2 ml solvent.

The volatile selenium compounds in aqueous extracts from the carbon were fractionated by anion-exchange chromatography on 100 cm by 1 cm columns of Dowex 1-X8 in the sulphate form, using 1·25N sulphuric acid as the eluting agent (Asher, Evans, and Johnson 1967). Organic extracts were analysed on an Aerograph Hi-Fi-600C analytical gas chromatograph (Wilkins Instrument & Research, Inc.) connected to a Speedomax H chart recorder (Leeds & Northrup) fitted with a disk integrator (Disc Instruments, Inc.). Good separations of the compounds present in the extracts were obtained on both a Carbowax column (10 ft by ½ in., 20% Carbowax 20 M on 60/80 Chromosorb W, coated with hexamethyldisilizane), and a polymethaphenylether column (5 ft by ½ in., 20% on 60/80 Chromosorb W, coated with hexamethyldisilizane). Electron-capture and hydrogen-flame-ionization detectors were used, depending on the solvent used to extract the volatile selenium compounds.

Dimethyl selenide (CH$_3$Se-CH$_3$) and dimethyl diselenide (CH$_3$Se-CH$_3$) were prepared for chromatographic standards by the method of Bird and Challenger (1942). Methaneselenol (CH$_3$SeH) was prepared by the method of Coates (1953). All compounds were purified on an Aerograph A-90-P preparative gas chromatograph (Wilkins Instrument & Research, Inc.). Some extracts containing plant volatiles were also fractionated on the preparative gas chromatograph using a 10 ft by ½ in. polymethaphenylether column. Fractions were collected manually, the exit tube dipping into vials containing either carbon disulphide at approximately 0°C or cyclohexane at 7°C.

Extracts and fractions were counted for $^{78}$Se activity on a Tracerlab SC-57A deep-well counter with stepwise scanning spectrometer, automatic sample changer, and print-out. The radioactivity collected was shown to be authentic $^{78}$Se by γ-ray spectrometry with the same instrument.

III. Results

In both the high- and low-selenium treatments, volatile selenium compounds were released both by intact plants and by oven-drying tops or roots. Consistently more $^{78}$Se was trapped from harvested plants drying for 24 or 36 hr in an oven at 70°C than from intact living plants over a 4- or 5-day collection period. In both selenium treatments 3–7% of the total $^{78}$Se in the tops was trapped in the form of volatile compounds on oven-drying. With the low-selenium treatment this represented approximately $10^{-8}$ moles Se per culture compared with $10^{-6}$ moles per culture from the high-selenium treatment.

Several solvents were found to be effective in removing volatile selenium compounds from the active carbon. In the high-selenium treatment, a single
extraction with acetone removed 56% of the selenium compounds from the carbon (Fig. 1). Benzene or chloroform removed a similar amount. Carbon disulphide, diethyl ether, or ethanol also removed substantial amounts of the selenium compounds but cyclohexane dissolved a total of only 6% in four consecutive extractions. The amount of selenium extracted by water was intermediate between cyclohexane and the other organic solvents.

Fig. 1.—Solvent extraction of 75Se activity from carbon used to trap volatile selenium compounds from A. racemosus.

Anion-exchange chromatography of aqueous extracts (Asher, Evans, and Johnson 1967) revealed the presence of two selenium compounds (Fig. 2). The chromatographic behaviour of these compounds suggested that they were the same two volatile compounds which had been isolated in previous experiments with lucerne (Asher, Evans, and Johnson 1967).
Of the organic solvents which removed more than 20% of the selenium in the first extraction, all except carbon disulphide gave inconveniently large signals with the hydrogen-flame-ionization detector of the analytical gas chromatograph. Similarly, except for cyclohexane and ether, each of the organic solvents tested gave inconveniently large signals with the electron-capture detector. Consequently, carbon disulphide extracts were analysed using the flame detector, and cyclohexane or ether extracts were analysed using the electron-capture detector.

Figure 3 shows the gas chromatographic separation of volatile compounds collected from oven-drying tops of plants from high- and low-selenium treatments. Peaks 1 and 2 were multiple peaks which could be further resolved at lower column temperatures. Peaks 1–6 and peak 8 were common to both selenium treatments, although the area under peak 5 was much greater with extracts from the high-selenium treatment. Peak 7 could be detected only in the extracts from the high-selenium treatment. The array of volatile compounds collected from oven-drying roots was closely similar to that collected from the tops.

The preparative gas chromatograph was used to isolate peaks 5 and 7 from the other volatile compounds present in carbon disulphide extracts obtained from the high-selenium treatment. The peaks present in each of the resultant fractions were then measured on the analytical gas chromatograph. Fractions containing peaks 1–4 contained less than 1% of the total $^{75}$Se activity recovered (recovery on the preparative instrument was about 80%). Virtually all of the activity recovered was found in fractions containing peak 5 or peak 7. Fractions containing only peaks 6 or 8 contained no detectable activity.
The small amounts of $^{75}$Se present in fractions containing peaks 1 and 2 and the absence of a peak corresponding with dimethyl selenide (retention time 1 min) indicate that dimethyl selenide was either absent from the carbon disulphide extracts or present in only trace amounts (Fig. 3). Experiments with pure dimethyl selenide, and other monoselenides, showed that the active carbon used to trap volatiles of plant origin was highly effective in trapping monoselenides and that these monoselenides could be readily extracted with carbon disulphide. Hence it seems reasonable to assume that dimethyl selenide would have been detected in this experiment if it had been produced by the *Astragalus* plants.

In fractions containing only peak 7 there was a linear relationship between the amount of $^{75}$Se in each fraction and the corresponding area under the peak, i.e. the amount of the volatile compound present. The $^{75}$Se activity associated with peak 7 varied from 59 to 84% of the total activity recovered.

A comparison of the chromatographic behaviour of peak 7 with that of known alkyl selenides and diselenides suggested that peak 7 might be dimethyl diselenide [Fig. 3(c)]. This was confirmed by co-chromatography of peak 7 and pure dimethyl diselenide on the analytical gas chromatograph, using both the polymetaphenylether and the Carbowax columns.

Although it was comparatively easy to obtain fractions containing only dimethyl diselenide (peak 7), fractions containing only peak 5 could not be obtained. Thus the peak 5 fractions were always contaminated with some dimethyl diselenide (peak 7). When allowance was made for the $^{75}$Se activity contributed by this dimethyl diselenide, the relationship between $^{75}$Se activity and the area under
peak 5 in various fractions was linear. The total $^{75}$Se associated with peak 5 was found to vary from 15 to 40% of the activity recovered. The retention time of peak 5 did not correspond with that of any known alkyl monoselenide or diselenide (Evans and Johnson 1966), nor with that of methaneselenol, a volatile compound which is easily oxidized to dimethyl diselenide.

Initial attempts to demonstrate the presence of either dimethyl diselenide or peak 5 in carbon disulphide extracts obtained from intact plants were unsuccessful, presumably because the amounts of these compounds present were too small to detect with the flame-ionization detector. Previous work had shown that the electron-capture detector was about 130 times as sensitive to dimethyl diselenide as the flame-ionization detector (Evans and Johnson 1966). When cyclohexane or ether extracts were analysed using the electron-capture detector the presence of both dimethyl diselenide and peak 5 were clearly demonstrated (Fig. 4).

Comparisons of the areas under peak 5, using the electron-capture detector, with the corresponding areas obtained using the flame-ionization detector showed that the electron-capture detector was more sensitive to that compound by a factor of about 30.

IV. DISCUSSION

In the present study, oven-drying *A. racemosus* released 10–100 times as much volatile selenium per culture as was obtained in a previous study with lucerne (Asher, Evans, and Johnson 1967). However, the amounts of volatile selenium collected were still too small to permit the isolation and identification of the component compounds by classical chemical methods. Instead, differences in solubility and in chromatographic behaviour were used to separate the components, and attempts were made to identify individual compounds by comparing their chromatographic behaviour with those of pure reference compounds.

Of the four compounds isolated in this study all were readily soluble in carbon disulphide. Two of them were also readily soluble in water, but insoluble in ether. Anion-exchange chromatography of aqueous extracts suggested that these two compounds were the same as those previously isolated from lucerne (Asher, Evans, and Johnson 1967). Attempts to characterize these compounds by gas chromatography have been unsuccessful so far.

The remaining two compounds were readily soluble in ether and slightly soluble in cyclohexane. One of them, dimethyl diselenide, is insoluble in water. The other (peak 5) appeared to be slightly soluble in water since, when organic extracts containing it were shaken with water, some of the $^{75}$Se activity was transferred to the aqueous phase. Anion-exchange chromatography showed that this $^{75}$Se activity was not due to the presence of either of the volatile selenium compounds previously designated as soluble in water. The apparent water-solubility of peak 5 may explain the fact that the sum of the water-soluble and ether-soluble fractions exceeded 100% after the fourth extraction (Fig. 1).

In contrast with results obtained in experiments with lucerne, the ether-soluble component of the volatile selenium compounds from *A. racemosus* was relatively large. Thus, a single extraction with ether removed more than 35% of the selenium
from the carbon, compared with less than 10\% for lucerne. The smaller proportion of ether-soluble volatiles in lucerne, together with the smaller total yield of volatile selenium compounds, may explain the fact that neither peak 5 nor dimethyl diselenide were detected when carbon disulphide extracts from lucerne were analysed by gas chromatography using the flame-ionization detector. It is suggested that the greater sensitivity of the electron-capture detector for these compounds should make feasible a re-examination of the ether-soluble volatiles from lucerne (Asher, Evans, and Johnson 1967). Further research is also needed to determine the identity of the two water-soluble volatile selenium compounds released by both lucerne and A. racemosus, and to identify the ether-soluble selenium compound corresponding with peak 5 in the present study.

Studies with animals (Petersen, Klug, and Harshfield 1951; McConnell and Portman 1952; McConnell and Roth 1966) and with fungi (Dransfield and Challenger 1955) have shown that these organisms are capable of synthesizing dimethyl selenide, and it has been suggested that this compound might be responsible for the characteristic odour of Astragalus plants high in selenium (Rosenfeld and Beath 1964; Virupaksha and Shrift 1965). However, in the present study, while dimethyl diselenide was found to be produced in appreciable quantities by A. racemosus, no evidence for the production of dimethyl selenide or any other alkyl monoselenide was obtained.

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