

THE SEPARATION OF SOME ALKYL SELENIUM COMPOUNDS BY GAS CHROMATOGRAPHY

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Many organic sulfur compounds have been identified in plant tissues by gas-liquid chromatography^{1,2}. As selenium forms many compounds analogous to those formed by sulfur, this study was made to determine the chromatographic characteristics of some organic selenium compounds which might prove useful as reference compounds in the identification of selenium metabolites in plant tissues.

EXPERIMENTAL

Preparation of compounds

Dimethyl, diethyl and di-*n*-propyl selenides were prepared essentially by the method of BIRD AND CHALLENGER³. Dimethyl, diethyl and di-*n*-propyl diselenides were prepared according to the method of TSCHUGAEFF AND CHLOPIN⁴, as modified by BIRD AND CHALLENGER³. Ethyl selenocyanate was prepared by the method of WEAVER AND WHALEY⁵, from potassium selenocyanate prepared according to CAMPBELL AND McCULLOUGH⁶. All methods of preparation were modified to semimicro scale, to give yields of approximately 1 g of each compound. All preparations were made inside a polyethylene glove box, within a well ventilated hood to prevent the spread of the unpleasant odors. Purification of all products was achieved by preparative gas chromatography resulting in virtually 100% purity as indicated by further analytical gas chromatographic studies.

Carbon disulfide and cyclohexane were used as solvents for the compounds, depending on which detector system was in use. All compounds were completely soluble in both solvents.

Apparatus

The preparative gas chromatograph used in the purification of the synthesized compounds was a Wilkens Instrument and Research, Inc. Model, Aerograph A-90-P, connected to a Leeds and Northrup, Speedomax H, Type S recorder with a disc chart integrator (Disc Instruments, Inc. Model 207). Samples were collected manually. The column used for all purifications was 5 ft. \times 1/4 in., Silicone Fluid (Methyl) SF-96 on 60/80 firebrick, originally supplied with the instrument. Helium was used as carrier gas with a flow rate of 35-40 cc/min.

The analytical gas chromatograph used for the separation of the organic selenium compounds was a Wilkens Instrument and Research, Inc., Hi-Fi 600C, with

either a hydrogen flame ionization detector or an electron capture detector, using the same recorder as was used on the preparative instrument.

10 μ l Hamilton microsyringes were used for all sample injections.

Columns

The three columns used in the Hi-Fi 600C (5 ft. \times $\frac{1}{8}$ in., 20 % Polymetaphenylether (5-ring) on 60/80 Chromosorb W coated with hexamethyldisilazane (HMDS); 10 ft. \times $\frac{1}{8}$ in., 20 % Carbowax 20 M on 60/80 HMDS Chromosorb W; 10 ft. \times $\frac{1}{8}$ in., 20 % Silicone Oil DC550 on 60/80 Chromosorb W coated with dimethyldichlorosilane (DMCS)) were supplied by Wilkens Instrument and Research, Inc. Nitrogen was used as carrier gas, at a flow rate between 20 and 30 cc/min. All flow rates were measured with a soap bubble flowmeter at the column exit.

Procedure

Using the hydrogen flame ionization detector, the retention times of the alkyl selenium compounds were determined on each of the three columns at column temperatures within the range of 35–175°. The injector temperature was set at 50 to 100° higher than the column temperature. One per cent solutions of each selenium compound in carbon disulfide were used for these determinations, as carbon disulfide gives very little response with this detector system.

For the determination of the ϕ value for each compound, defined as the ratio of response given by the electron capture detector to that given by the hydrogen flame ionization detector², $\phi = \text{EC/FL}$, peak areas were measured with the disc chart integrator. Injections of pure alkyl monoselenides, and solutions of alkyl diselenides and ethyl selenocyanate in cyclohexane were used for determinations with the electron capture detector.

RESULTS AND DISCUSSION

Tables I, II and III give the retention times for each compound on the three columns used. A comparison of the degree of resolution of the organic selenium compounds which was obtained from each of the three columns, showed that the best resolution was achieved on the Polymetaphenylether column, but both Carbowax 20M and Silicone Oil DC550 columns gave satisfactory resolution of all the alkyl selenium compounds studied. However, the DC550 column was unsatisfactory for use above 150°, as a large amount of continuous column bleeding occurred above this temperature. Two additional columns, Silicone gum rubber SE30 on 60/80 DMCS Chromosorb W, and Apiezon L on 60/80 DMCS Chromosorb W, were unsuccessfully tried for the separation of these compounds. The SE30 column caused long tailing of all the peaks, and the Apiezon L column gave very broad peaks.

For each column, a logarithmic plot of retention time *vs.* the number of carbon atoms per compound gave straight line plots for the alkyl monoselenides and alkyl diselenides (Fig. 1). Similarly, logarithmic plots of retention time *vs.* boiling point gave straight line relationships for both the mono and the diselenides. There was no evidence for the formation of decomposition products from any of the compounds injected.

Figure 2 shows the complete resolution of the 7 alkyl selenium compounds on

TABLE I

RETENTION TIMES OF ALKYL SELENIUM COMPOUNDS ON THE POLYMETAPHENYLETHER COLUMN
N₂ carrier gas flow rate: 25 cc/min.

<i>Column temperature</i>	<i>Retention time (min)</i>					
	50°	75°	100°	125°	150°	175°
<i>Injector temperature</i>	100°	180°	180°	180°	225°	220°
Dimethyl selenide	2.3	1.4	0.8	0.6	0.5	
Diethyl selenide	12.0	5.8	2.4	1.8	1.0	
Dipropyl selenide	35.0	20.0	8.4	4.3	2.2	
Dimethyl diselenide		22.5	10.2	5.2	2.7	2.0
Diethyl diselenide			28.5	12.6	6.0	4.2
Dipropyl diselenide				35.5	12.4	9.0
Ethyl selenocyanate		30.5	11.5	7.0	3.2	2.7

TABLE II

RETENTION TIMES OF ALKYL SELENIUM COMPOUNDS ON THE CARBOWAX 20M COLUMN
N₂ carrier gas flow rate: 20 cc/min.

<i>Column temperature</i>	<i>Retention time (min)</i>					
	35°	45°	70°	100°	120°	160°
<i>Injector temperature</i>	100°	100°	180°	180°	180°	220°
Dimethyl selenide	7.0	4.0	2.8	1.7	1.3	0.8
Diethyl selenide		16.0	6.7	3.8	2.5	1.4
Dipropyl selenide			16.2	9.0	5.2	2.3
Dimethyl diselenide			36.5	16.0	8.9	3.8
Diethyl diselenide				32.0	14.7	6.4
Dipropyl diselenide				72.0	30.0	11.0
Ethyl selenocyanate				48.0	19.5	6.5

TABLE III

RETENTION TIMES OF ALKYL SELENIUM COMPOUNDS ON THE SILICONE OIL DC550 COLUMN
N₂ carrier gas flow rate: 25 cc/min.

<i>Column temperature</i>	<i>Retention time (min)</i>		
	100°	125°	150°
<i>Injector temperature</i>	180°	180°	225°
Dimethyl selenide	5.2	3.7	2.5
Diethyl selenide	15.5	9.2	5.8
Dipropyl selenide		28.0	14.0
Dimethyl diselenide		28.0	13.0
Diethyl diselenide			29.0
Ethyl selenocyanate		27.5	14.0

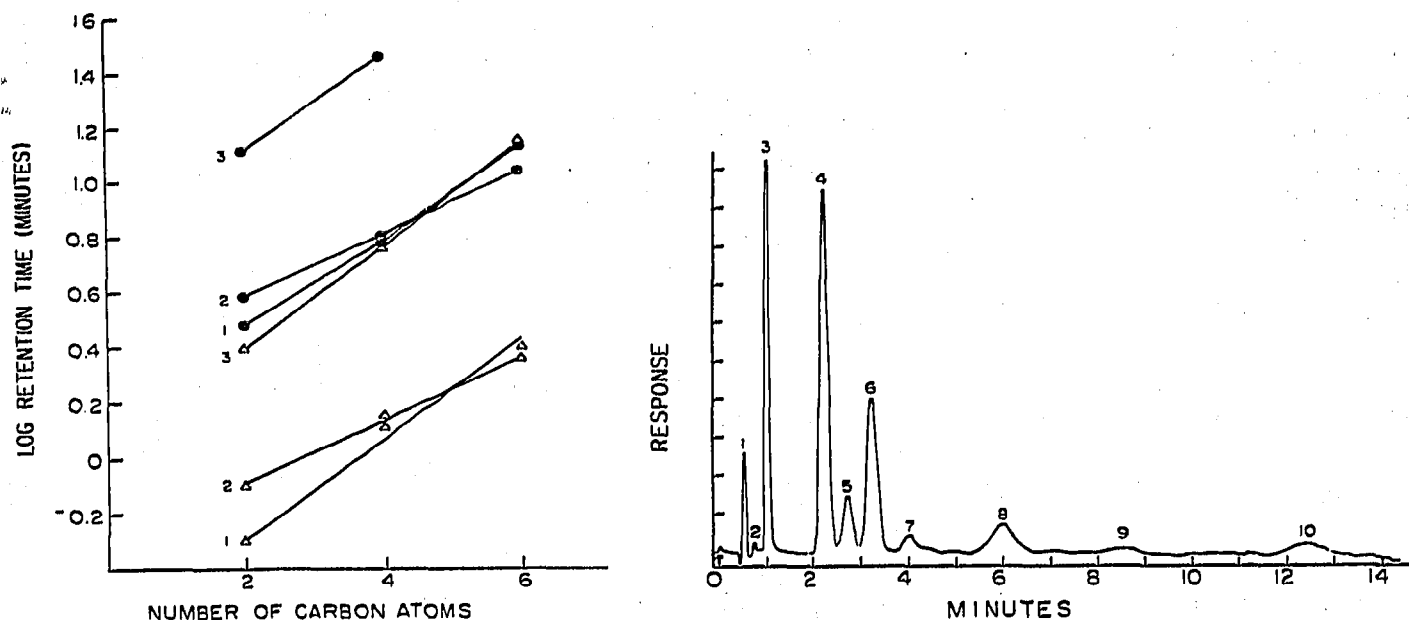


Fig. 1. The relationship between log retention time and number of carbon atoms. (1) Polymetaphenylether column at 150°; (2) Carbowax 20M column at 160°; (3) Silicone Oil DC550 column at 150°. (Δ — Δ) = alkyl selenides; (\bullet — \bullet) = alkyl diselenides.

Fig. 2. The separation of alkyl selenium compounds on a Polymetaphenylether column, with hydrogen flame ionization detector. Column temperature: 150°; injector temperature: 225°; N_2 carrier gas flow rate: 25 cc/min; (— \rightarrow) = injection point at time 0 min. 1% solution of each compound in carbon disulfide. (1) Dimethyl selenide; (2) carbon disulfide; (3) diethyl selenide; (4) dipropyl selenide; (5) dimethyl diselenide; (6) ethyl selenocyanate; (7) methyl ethyl diselenide?; (8) diethyl diselenide; (9) ethyl propyl diselenide?; (10) dipropyl diselenide.

the Polymetaphenylether column, at column temperature of 150°. Two additional peaks occurred at retention times 4.0 min and 8.2 min, which were tentatively identified as methyl ethyl diselenide, and ethyl propyl diselenide on the basis of their correspondence with the theoretical retention times of these compounds as determined from the logarithmic plot of retention time *vs.* number of carbon atoms in the alkyl diselenides. Similarly, the logarithmic plot of retention time *vs.* boiling point suggested that the additional peaks corresponded to methyl ethyl diselenide, and ethyl propyl diselenide. Absolute confirmation of this awaits the synthesis of the pure compounds. It appears that these mixed alkyl diselenides could have been formed in the mixture by interaction between dimethyl, diethyl and dipropyl diselenides.

TABLE IV

DETECTOR RESPONSE RATIOS

Compound	Approximate ϕ value
Dimethyl selenide	0.01
Diethyl selenide	0.002
Dipropyl selenide	0.001
Dimethyl diselenide	130.0
Diethyl diselenide	135.0
Dipropyl diselenide	150.0
Ethyl selenocyanate	320.0

The ϕ values determined for each compound are given in Table IV. They are approximate values, due to the difficulty in exactly reproducing the same sample size and conditions when using the two different detectors, and also due to the very great difference in response size with the two detectors. The largest response to the monoselenides was obtained with the hydrogen flame ionization detector, whereas the diselenides and ethyl selenocyanate gave the largest responses with the electron capture detector.

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SUMMARY

The following organic selenium compounds were separated by gas chromatography: dimethyl, diethyl and di-*n*-propyl selenides; dimethyl, diethyl and di-*n*-propyl diselenides; ethyl selenocyanate.

Three columns used for this study, Polymetaphenylether, Carbowax 20M and Silicone Oil DC550 on Chromosorb W, gave satisfactory separation of the organic selenium compounds injected, the best separation being achieved with the Polymetaphenylether column. All compounds were fully characterized on each of the columns with regard to determination of retention times at several different column temperatures. The ϕ value, reflecting the ratio in response given by the electron capture detector to that given by the hydrogen flame ionization detector, is presented for each compound.

REFERENCES

- 1 J. F. CARSON AND F. F. WONG, *J. Agr. Food Chem.*, 9 (1961) 140.
- 2 D. M. OAKS, H. HARTMAN AND K. P. DIMICK, *Anal. Chem.*, 36 (1964) 1560.
- 3 M. L. BIRD AND F. CHALLENGER, *J. Chem. Soc.*, (1942) 570.
- 4 L. TSCHUGAEFF AND V. G. CHLOPIN, *J. Russ. Phys. Chem. Soc.*, 47 (1915) 364.
- 5 W. E. WEAVER AND W. M. WHALEY, *J. Am. Chem. Soc.*, 68 (1964) 2115.
- 6 T. W. CAMPBELL AND J. D. MCCULLOUGH, *J. Am. Chem. Soc.*, 67 (1945) 1965.

J. Chromatog., 21 (1966) 202-206