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## GAS CHROMATOGRAPHIC AND MASS SPECTROMETRIC PROPERTIES OF ETHYL ESTERS OF DITHIOCARBAMIC ACIDS OF PRIMARY AND SECONDARY AMINES

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### SUMMARY

A method has been devised for determining primary and secondary amines in the form of dithiocarbamic acid ethyl esters by high-resolution gas chromatography and mass spectrometry. The dithiocarbamic acid ethyl ester derivatives of primary amines decompose by pyrolysis in the injection port of a chromatograph to isothiocyanates, whereas derivatives of secondary amines do not decompose. The mechanism of isothiocyanate formation from dithiocarbamic acid esters was established by identifying the pyrolysis products. This method permits simultaneous differentiation and determination of primary and secondary amines.

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### INTRODUCTION

Analysis of amines is highly important in structural and biochemical investigations and in the study of food products and pollutants. Free amines can be analysed by gas chromatography (GC) under appropriate conditions. Better results are often obtained in chromatographic separations by using suitable derivatives<sup>1</sup>.

Chromatographic analyses of acetyl<sup>2-4</sup>, trimethylsilyl<sup>5</sup>, trifluoroacetyl<sup>6</sup>, pentafluoropropionyl<sup>7</sup> and heptafluorobutyryl amine derivatives have been described. Some important primary amines have also been identified by using isothiocyanates via GC<sup>9,10</sup> or GC-mass spectrometry<sup>11</sup>.

Isothiocyanates can be obtained by thermal decomposition of carboalkoxy-dithiocarbamates<sup>12</sup>, a method we have previously checked<sup>13</sup>.

Dithiocarbamic acid ethyl esters are sufficiently volatile and thermostable for direct mass spectrometric analysis<sup>14,15</sup>. This paper reports the use of these esters as derivatives of the corresponding amines for the chromatographic analysis with capillary columns.

## EXPERIMENTAL

Commercially produced amines were used (Fluka, Buchs, Switzerland).

The dithiocarbamic acid ethyl esters, derivatives of primary and secondary amines, were obtained either in aqueous solution or in anhydrous medium.

To a mixture containing  $10 \mu M$  of each of the 11 amines investigated, dissolved in 2 ml of water, was added 110  $\mu l$  of carbon disulphide and 110  $\mu M$  of sodium hydroxide (110  $\mu l$  of 1 *N* NaOH). The mixture was shaken for 3 h, then following addition of 100  $\mu M$  of ethyl bromide, the shaking was continued for one more hour. After termination of the reaction the product was extracted with 5 ml of ethyl acetate. The organic fraction was washed with 0.5 ml of 1 *N* hydrochloric acid solution, then with water, and dried with anhydrous magnesium sulphate. The solution was concentrated in a dry nitrogen stream to a volume of 500  $\mu l$ . Chromatographic analysis was performed using 1–2  $\mu l$  of the solution.

The synthesis of dithiocarbamic acid ethyl esters in anhydrous medium was performed as described above, using chloroform as solvent and excess triethylamine instead of sodium hydroxide. The chloroform was evaporated under vacuum and the residue treated as above.

The carboethoxydithiocarbamates of the corresponding amines were obtained in a similar manner, except for the addition of ethyl chloroformate instead of ethyl bromide in the second reaction stage.

### *Gas chromatography*

Chromatographic analysis was performed by using a capillary column coated with SE-30 (ref. 16) or polar Silar 5CP (ref. 17). The efficiency of columns coated with SE-30 containing Silanox-101, determined for *n*-tetracosane at 250° with a column 60 m  $\times$  0.025 cm I.D. was 180,000.

The capillary column coated with Silar 5CP was prepared according to the two-step dynamic/evaporative method<sup>17</sup>. The efficiency of the Silar 5CP column (40 m  $\times$  0.03 cm I.D.), determined for methyl linoleate at 175°, was 90,000.

Other analytical conditions have been described earlier<sup>18</sup>.

### *Mass spectrometry*

Mass spectra were measured with a Varian MAT 711 mass spectrometer linked with a Varian 620/L-100 computer. A Varian Aerograph 1400 gas chromatograph adjusted to the work with a capillary column was attached to the mass spectrometer through a Biemann-Watson separator. The capillary column was attached to the chromatograph by using a splitting injector at 1:10 ratio and the so-called make-up system at the column end (20 ml helium). The efficiency of a 30-m long SE-30 capillary column, as measured in the gas chromatograph (flame ionization detector) for tridecane at 120°, was 120,000 theoretical plates. The gas chromatogram obtained from the total ionization current recorded under similar conditions as before and at a separator temperature of 250°, had an efficiency of 70,000 theoretical plates (58%). Moreover, the shape of the chromatographic peak recorded as total ion current was asymmetrical, this pointing to the adsorption effects occurring in the Biemann-Watson separator.

The temperature of the ion source was 260° and that of the molecular separa-

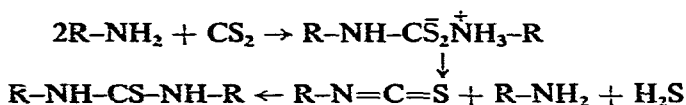
tor 250°. An ionization energy of 20 eV was used. The scanning rate from  $m/e$  30 to 300 was 4 sec/dec.

### Pyrolysis

Pyrolysis of the dithiocarbamic acid ethyl esters derived from primary amines was performed with a Chemical Data Systems pyrolyser connected to the injection port of a gas chromatograph equipped with a Chromosorb 102 column (60–80 mesh; Applied Science Labs., State College, Pa., U.S.A.). The platinum band of the pyrolyser was covered with a sample of the substance investigated dissolved in acetone. The temperature was increased by 20°/msec to a maximum of 300°. The helium flow-rate during pyrolysis was 20 ml/min. The mass spectra for the chromatographic peaks were registered for 20 eV ionization energy.

### RESULTS AND DISCUSSION

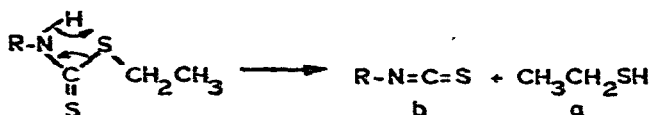
To obtain isothiocyanates by the action of carbon disulphide it is necessary to use an alkaline medium to prevent a side reaction which makes quantitative determination of amines impossible owing to the formation of the following products<sup>19</sup>.



The use of a very large excess of carbon disulphide gives rise to a very small amount of the thiourea derivative.

Isothiocyanates can be obtained quantitatively by pyrolysis of carboalkoxy-dithiocarbamates<sup>12,13</sup>. They can also be formed by pyrolysis of dithiocarbamic acid ethyl esters, as demonstrated in the present study. It is this type of amino acid derivative that we have used in identifying amino acids by mass spectrometry<sup>14,15</sup>.

We have found that, when dithiocarbamic acid ethyl esters derived from primary amines are subjected to chromatographic analysis, they decompose by pyrolysis in the injection port of the chromatograph to the corresponding isothiocyanates. The gas chromatogram (Fig. 1) of pyrolysis products of the dithiocarbamic acid ethyl ester of isopentylamine shows the presence of two products. On the basis of their mass spectra they could be identified as thioethanol (Fig. 2) and isopentyl isothiocyanate (Fig. 3). The presence of these two products suggests the following pyrolysis mechanism.



Such a course of reaction indicates that the dithiocarbamic acid ethyl esters of primary amines are suitable derivatives for chromatographic analysis.

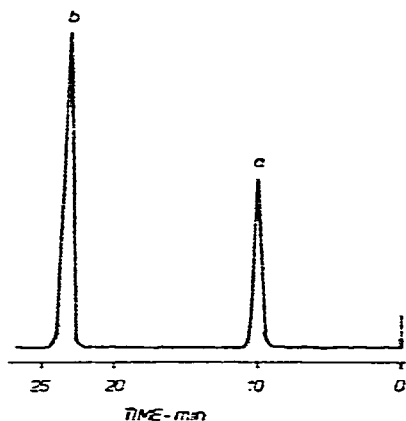


Fig. 1. Gas chromatogram of products of ethyl isopentyl dithiocarbamate pyrolysis. Column: 2 m  $\times$  0.2 cm Chromosorb 102; initial temperature: 100°; temperature programme; 4°/min.

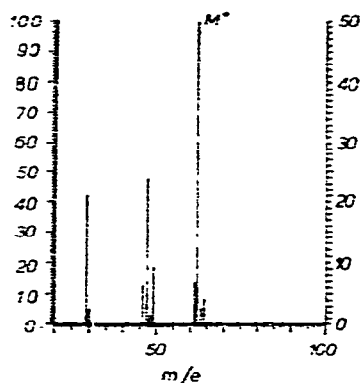


Fig. 2. Mass spectrum of GC peak a in Fig. 1.

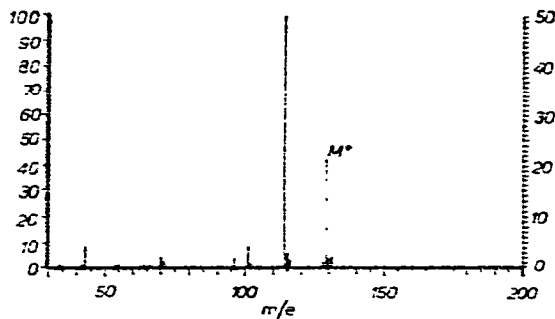
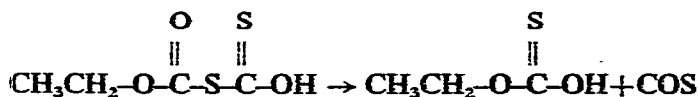


Fig. 3. Mass spectrum of GC peak b in Fig. 1.

Chromatographic analysis of a mixture of dithiocarbamic acid esters in aqueous medium in the presence of sodium hydroxide reveals a by-product with a shorter retention time than that of benzyl isothiocyanate (Fig. 4). Chromatographic analysis of amines is hampered by that by-product. On the basis of its mass spectrum (Fig. 5) we have ascribed to it the structure of trithiocarbonic acid ethyl diester,  $\text{CH}_3\text{CH}_2\text{-S-C(S)-S-CH}_2\text{CH}_3$ . For that compound to be formed, the solution must contain the  $\text{CS}_3^{2-}$  ion<sup>20</sup>, which yields the above ester with ethyl bromide.

The gas chromatogram of a mixture of isothiocyanates formed by pyrolysis of carboalkoxydithiocarbamates in aqueous medium also reveals the presence of a by-product which elutes immediately after elution of the cyclohexyl isothiocyanate. The structure of that by-product has been determined on the basis of its mass spectrum (Fig. 6). It is thiocarbonic acid ethyl monoester,  $\text{CH}_3\text{CH}_2\text{-O-C(S)-OH}$ , which presumably forms in the injection port according to the reaction



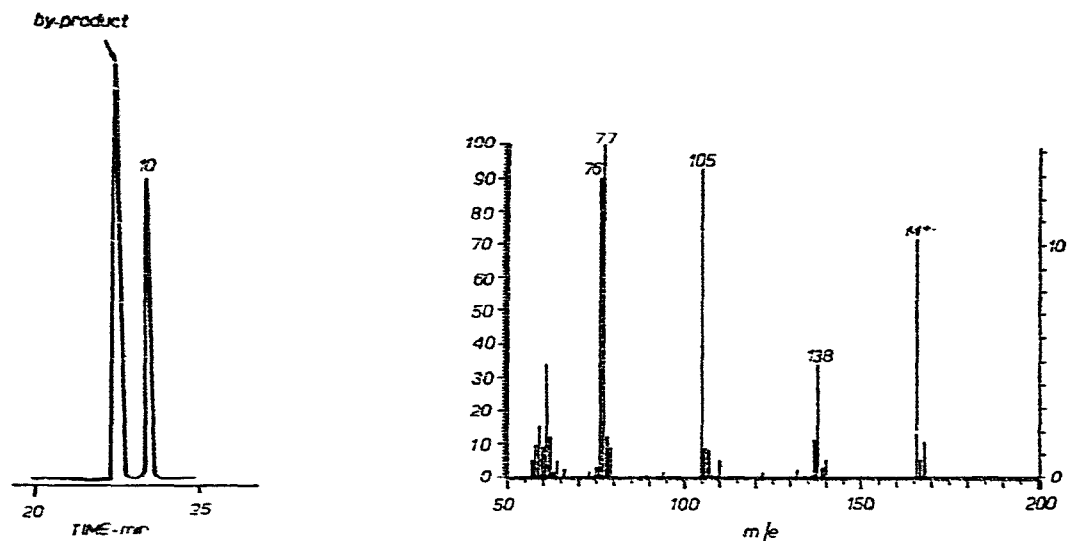


Fig. 4. Gas chromatogram of by-products arising during preparation of dithiocarbamic acid ethyl esters in aqueous medium. Column: 48 m  $\times$  0.03 cm SE-30; initial temperature: 70°; temperature programme: 4°/min.

Fig. 5. Mass spectrum of by-products obtained in water procedure of dithiocarbamic acid ethyl ester preparation.

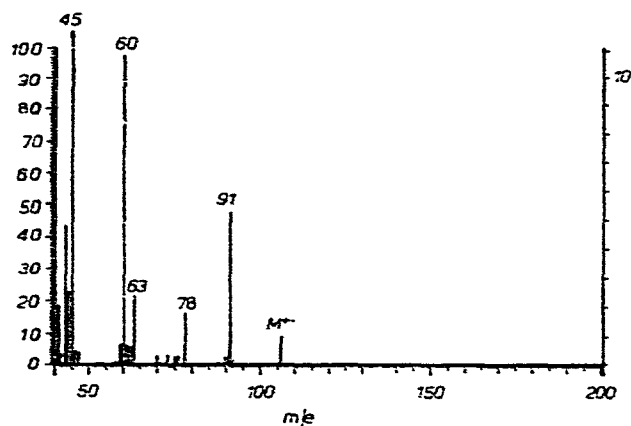


Fig. 6. Mass spectrum of by-products obtained in water procedure of carboalkoxydithiocarbamate preparation.

The occurrence of the by-products described above prompted us to devise suitable conditions for obtaining the corresponding derivatives in non-aqueous medium. Both by-products characterized above form only in basic aqueous medium. The use of chloroform as solvent therefore prevented their formation.

Separation of the mixture of the isothiocyanates formed by pyrolysis of the dithiocarbamic acid ethyl esters derived from primary amines, in the injection port of the gas chromatograph, is shown in Fig. 7 (SE-30) and in Fig. 8 (Silar 5CP).

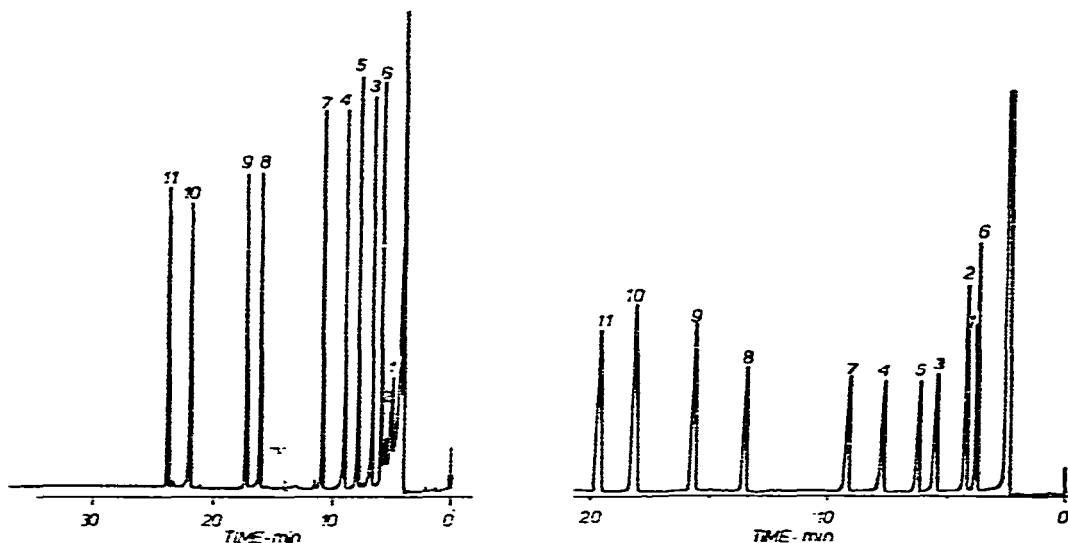


Fig. 7. Gas chromatogram of a mixture of dithiocarbamic acid ethyl ester derivatives of primary amines. Numbers identified in Table I. Conditions as in Fig. 4.

Fig. 8. Gas chromatogram of a mixture of dithiocarbamic acid ethyl ester derivatives of primary amines. Numbers identified in Table I. Column: 45 m  $\times$  0.03 cm Silar 5CP; initial temperature: 60°; temperature programme: 4°/min.

Identical chromatograms were obtained for dithiocarbamic acid esters and for carbethoxydithiocarbamates. The dithiocarbamic acid ethyl esters are, however, more stable. The sequence of isothiocyanate elution has been determined by measuring mass spectra (Table I) or by using the coinjection method.

Dithiocarbamic acid ethyl esters derived from secondary amines do not decompose in the injection port of the chromatograph. Chromatograms of a

TABLE I  
PARTIAL ELECTRON IMPACT MASS SPECTRA OF RNCS

Number	R	$M^+$	$M-NCS^{1+}$	$CH_2NCS^{1+}$	Other $m/e$ ions
1	$CH_3$	73* (100)**	—	72 (3)	
2	$CH_3CH_2$	87 (100)	—	72 (2)	
3	$CH_3(CH_2)_2$	101 (100)	43 (4)	72 (2)	
4	$CH_3(CH_2)_3$	115 (100)	57 (8)	72 (4)	114 (11)
5	$(CH_3)_2CHCH_2$	115 (100)	57 (23)	72 (2)	
6	$(CH_3)_2C$	115 (100)	57 (28)	72 (4)	
7	$(CH_3)_2CH(CH_2)_2$	129 (42)	71 (3)	72 (2)	114 (100), 101 (7)
8	$CH_3(CH_2)_3$	143 (13)	—	—	115 (100), 110 (21), 101 (3)
9	$(CH_2)_3CH$	141 (100)	83 (20)	—	115 (2), 106 (6), 82 (38)
10	$C_6H_5CH_2$	149 (33)	91 (100)	—	92 (11)
11	$CH_3(CH_2)_7$	171 (2)	—	72 (2)	115 (100), 138 (52), 101 (3), 96 (8)

\* Indicates  $m/e$  value.

\*\* Figures in parenthesis refer to relative intensity of indicated ion peaks.

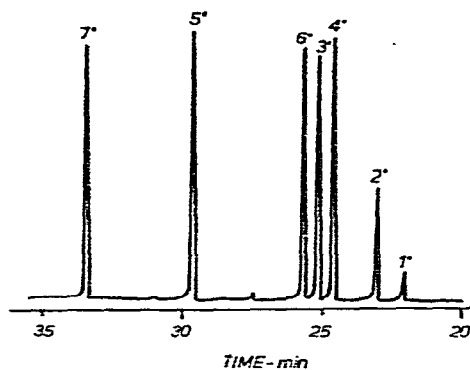
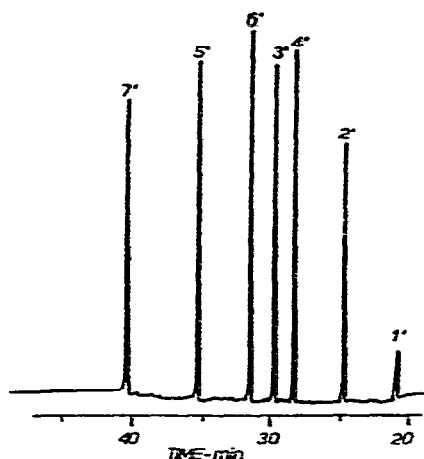


Fig. 9. Gas chromatogram of a mixture of dithiocarbamic acid ethyl ester derivatives of secondary amines. Numbers identified in Table II. Conditions as in Fig. 4.

Fig. 10. Gas chromatogram of a mixture of dithiocarbamic acid ethyl ester derivatives of secondary amines. Numbers identified in Table II. Conditions as in Fig. 8.

TABLE II

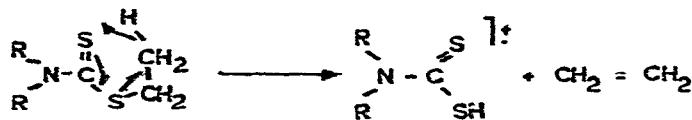
PARTIAL ELECTRON IMPACT MASS SPECTRA OF  $R_2NCSSCH_2CH_3$

Number	R	$M^{+\cdot}$	$M-C_2H_4^{+\cdot}$	$M-C_2H_4-SH^{+\cdot}$
1'	$CH_3$	149*(94)**	121 (59)	88 (100)
2'	$CH_3CH_2$	177 (95)	149 (100)	116 (35)
3'	$CH_3(CH_2)_2$	205 (100)	177 (84)	144 (21)
4'	$(CH_3)_2CH$	205 (100)	177 (26)	144 (23)
5'	$CH_3(CH_2)_3$	233 (100)	205 (88)	172 (34)
6'	$(CH_3)_2CHCH_2$	233 (100)	205 (92)	172 (21)
7'	$CH_3(CH_2)_5$	261 (100)	233 (58)	200 (38)

\* Indicates  $m/e$  value.

\*\* Figures in parenthesis refer to relative intensity of indicated ion peaks.

mixture of these derivatives are shown in Fig. 9 (SE-30) and in Fig. 10 (Silar 5CP). Their mass spectra (Table II) show the presence of abundant molecular ions and characteristic fragment ions. The abundant ions are related to the fragmentation of the ester residue of dithiocarbamic acid<sup>14</sup>. In the first step ethene is eliminated via a McLafferty rearrangement.



The second fragmentation step depends on the structure of the R group. The process of radical HS<sup>•</sup> elimination occurs for all amine derivatives.



For the diethylamine derivative, elimination of hydrogen sulphide is also observed.



The fragmentation processes described here are similar to those observed in the spectra of analogous amino acid derivatives<sup>14</sup>.

The essential differences in the properties of dithiocarbamic acid ethyl esters of primary and secondary amine derivatives can be used for distinguishing these compounds chromatographically, *i.e.* for their separation and identification. Chromatographic separation of a mixture of such amines is shown in Fig. 11.

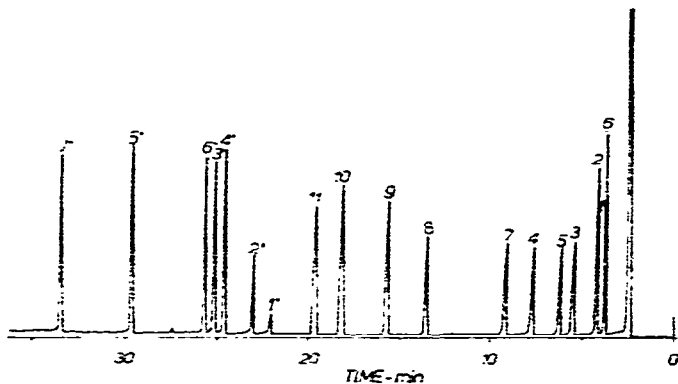


Fig. 11. Gas chromatogram of a mixture of dithiocarbamic acid ethyl esters of derivatives of primary and secondary amines. Conditions as in Fig. 8.

The detection level of isothiocyanates and dithiocarbamic acid ethyl esters derived from secondary amines was determined by means of a gas chromatograph for two selected compounds. Measurements were carried out on an SE-30 column (splitter assembly with a splitting ratio of 1:20). The chromatograms obtained for the isopentyl isothiocyanate (Fig. 12) and the di-*n*-butylamine dithiocarbamic acid ethyl ester (Fig. 13) show in both cases a detection level of 2 ng.

The use of dithiocarbamic acid esters for the identification of naturally occurring amines will be reported in a separate paper.



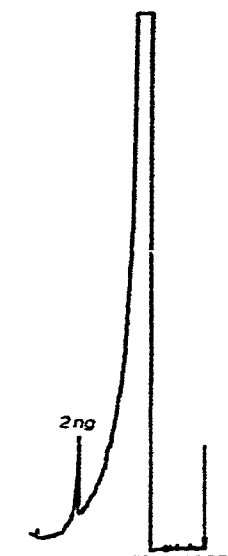


Fig. 12. Gas chromatogram showing the detectable amounts of isopentyl isothiocyanate. Column as in Fig. 4, temperature 100°.

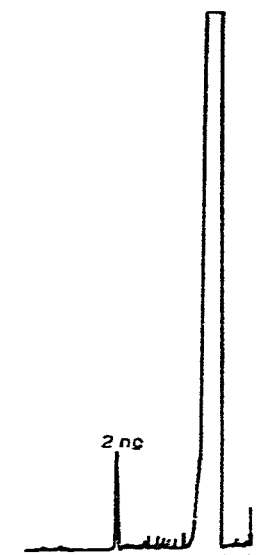


Fig. 13. Gas chromatogram showing the detectable amounts of ethyl di-*n*-butyl dithiocarbamate. Column as in Fig. 4, temperature 200°.

#### ACKNOWLEDGEMENT

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