# The Conversion of 2,4-Dinitrophenylhydrazine Derivatives of Monocarbonyls to Ethylenethioacetals and Ethylenethioketals for Analysis by Gas Liquid Chromatography

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## ABSTRACT

A method was developed for the direct conversion of 2,4-dinitrophenylhydrazones (2,4-DNPHS) of *n*-alkanals and methylketones into ethylenethioacetals and ethylenethioketals for analysis by gas liquid chromatography (GLC). The method depended on heating the 2,4-DNPHS and 1,2-ethanedithiol with boron fluoride ($BF_3$) ethyl ether at 125 C for 12 min or with *p*-toluenesulfonic acid at 200 C for 15 min. The reaction was extracted with pentane, and after evaporation, the product was dissolved in *o*-xylene and injected into the GLC. High yields of above 90% were obtained from long chain 2,4-DNPHS and somewhat lower yields of about 80% were obtained from short chain 2,4-DNPHS. Eight standard ethylenethioacetals and ethylenethioketals were synthesized; their elemental analysis, bp, mp, and retention times on FFAP and SE-30 GLC columns were determined.

## INTRODUCTION

In routine analysis of carbonyl compounds, the use of GLC is of great advantage. However, chromatographing the 2,4-dinitrophenylhydrazones (2,4-DNPHS), the widely used carbonyl derivative, is not easily done due to low volatility and decomposition at high temperature. Soukup et al. (1) injected a mixture of the derivatives directly into the GLC for qualitative analysis. Fracchia et al. (2) used three different conditions of column temperature and carrier gas flow rate to obtain optimum separation between short chain carbonyl derivatives. They also used this method for the quantitative determination of the derivatives. Other workers who have gas chromatographed 2,4-DNPHS include Galetto et al. (3), Leonard and Kiefer (4), and Shimizu et al. (5).

Many workers converted the 2,4-DNPHS into other carbonyl derivatives with better chromatographic properties. Dimethylacetals have been widely used for GLC determination of long chain aldehydes (6,7). This derivative, however, is limited to aldehydes and, furthermore, it is not stable on many GLC columns (8).

Stacy and coworkers (9) proved that thiols add to the azomethine linkage of Schiff-bases at room temperature and the addition was favored by the presence of an electron withdrawing group. They also found that in treating the thiol-Schiff-base adduct with acylating agents, various products, including the thioacetal of the original aldehyde, were produced. The presence of an electron-withdrawing group in the Schiff-base greatly accelerated the decomposition of the adduct.

The 2,4-DNPHS are Schiff-bases which contain two electron-withdrawing groups, and accordingly, the thiol-2,4-DNPHS adduct should decompose easily into the thioacetal or thioketal derivative.

When extracts or column fractions are analyzed for carbonyls, it is difficult to recover the carbonyls without loss due to volatilization. Consequently the carbonyls are recovered as stable derivatives such as the 2,4-DNPHS Separation and quantification of the 2,4-DNPHS using column and thin layer techniques can be tedious and laborious. On the other hand direct gas liquid chromatography (GLC) analysis of the 2,4-DNPHS is not easy due to the low volatility of these derivatives. In fact, quantification of 2,4-DNPHS using GLC is difficult at best and nearly impossible beyond hexanal. Therefore, we studied the conversion of 2,4-DNPHS to stable derivatives volatile enough to extend the range of analysis yet non-

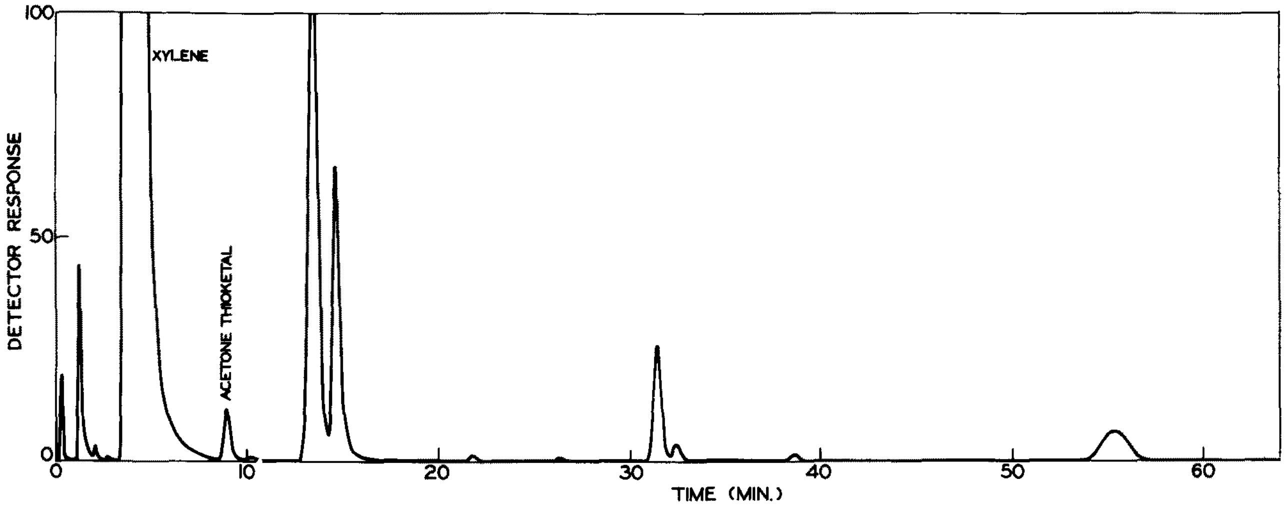


FIG. 1. A chromatogram of the extract of the reaction mixture of 2-propanone 2,4-DNPH with $BF_3$ as a catalyst, showing the impurity peaks.

TABLE I

Yields of Ethylenethioacetals and Ethylenethioketals Obtained From 2,4-DNPHS of *n*-Alkanals and Methyl Ketones Using $BF_3$ Ethyl Ether Catalyst

| 2,4-DNPHS[a] | Ethanedithiol[b] ml | $BF_3$[c] ml | Heat Treatment | Yield % |
|---|---|---|---|---|
| Ethanal- | 2.2 | 0.90 | 125 C/120 min | 75.0 |
| Hexadecanal- | 1.2 | 0.48 | 125 C/120 min | 92.0 |
| 2-Propanone- | 2.0 | 0.80 | 125 C/120 min | 85.0 |
| 2-Octanone- | 1.6 | 0.65 | 125 C/120 min | 91.0 |

[a] The hydrazones were dissolved in 0.3 ml $HCCL_3$.

[b] Ethanedithiol volume was equivalent to 300 times the moles of 2,4-DNPHS.

[c] $BF_3$ volume was equivalent to 80 times the moles of 2,4-DNPHS.

volatile enough so as not to be lost during concentration.

The purpose of this investigation was to study the conversion of 2,4-DNPHS into the corresponding ethylenethioacetal and ethylenethioketal and the use of these derivatives for carbonyl analysis by GLC.

## ANALYTICAL TECHNIQUES

### Chemicals

All carbonyl standards were obtained from commercial manufacturers. 1,2-Ethanedithiol was obtained from Aldrich. $BF_3$ ethyl ether was obtained from Fisher, *p*-toluenesulfonic acid (p-TSA) from Eastmen, and 2,4-dinitrophenylhydrazine from Aldrich. The above chemicals were of the highest purity available and were used without further purification. All solvents were purified to remove carbonyl impurities.

### Preparation of Standards

The 2,4-DNPHS were prepared according to the method of Shriner et al. (10). All derivatives were crystallized from ethyl alcohol until constant and sharp melting points were obtained.

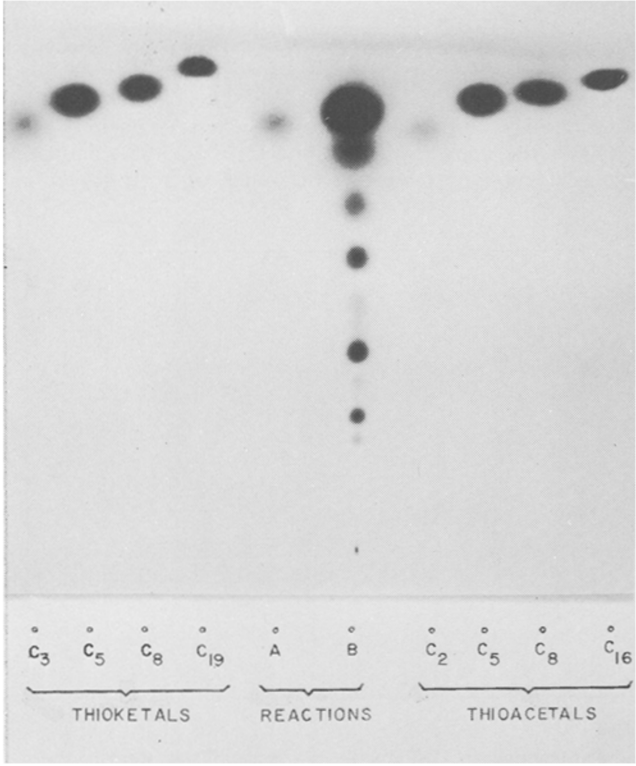


FIG. 2. A Silica Gel G thin layer plate showing ethylenethioacetal and ethylenethioketal standards and 2-propanone 2,4-DNPH reaction mixture extract using p-TSA catalyst (A) and $BF_3$ catalyst (B).

The ethylenethioacetal and ethylenethioketal standards were prepared by the method of Fieser (11). After washing the reaction mixture with alcoholic alkali (7.5 N NaOH in 50% methyl alcohol), the crystals obtained were recrystallized four times from methyl alcohol and dried over Drierite under vacuum. The oily derivatives were distilled two times under a 90 $\mu$ vacuum. The standards were analyzed for sulfur, hydrogen and carbon contents by Micro-Tech Laboratories, Inc. GLC was used for calculating the percentage of impurities, which appeared in the chromatogram of the standards, by dividing the areas of the impurity peaks by the standard area times one hundred. The boiling points of the liquid standards were determined by the micro-method described by Shriner et al (10) and were corrected to 760 mm pressure.

### Yield Determination

The yields were obtained from calibration curves (concentration vs. peak area), prepared with each experiment and for each individual carbonyl using *o*-xylene as a solvent. Peak area was calculated by multiplying peak height times the width at half peak height. The procedure for making these calibration curves and the reason for using *o*-xylene or other high boiling solvents for injecting these compounds was reported by Metwally et al. (12). Yield per cent was calculated by the equation

$$\frac{\text{mg thioacetal obtained}}{\text{theo. mg thioacetal}} \times 100.$$

All yields reported were the average of at least duplicate determinations.

Controls, excluding the 2,4-DNPHS, were prepared with each experiment, using the same conditions and the same reaction mixture.

### Gas Chromatography

An Aerograph 1520, equipped with dual columns and flame ionization detectors, was used. The nitrogen carrier

TABLE II

Yields of Ethylenethioacetals and Ethylenethioketals Obtained From 2,4-DNPHS of *n*-Alkanals and Methyl Ketones Using p-TSA Catalyst

| 2,4-DNPHS[a] | GLC column | Yield % |
|---|---|---|
| 20 mg Ethanal- | FFAP | 78 |
| Octanal- | FFAP | 82 |
| | SE-30 | 80 |
| Hexadecanal- | FFAP | 85 |
| | SE-30 | 86 |
| 20 mg 2-Propanone- | FFAP | 72 |
| 2-Octanone- | FFAP | 93 |
| | SE-30 | 92 |
| 2-Nonadecanone- | FFAP | 81 |

[a] The conditions were 0.3 g p-TSA (Eastman), 0.075 ml $H_2O$ and 200 C for 15 min.

TABLE III

Elemental Analysis, Boiling Points and Melting Points of Ethylenethioacetal and Ethylenethioketal Standards

| Sample | % C | | % H | | % S | | bp, C | mp, C |
|---|---|---|---|---|---|---|---|---|
| | Exper. | Theo. | Exper. | Theo. | Exper. | Theo. | | |
| Ethylenethioacetal of | | | | | | | | |
| Ethanal | 39.76 | 39.95 | 6.63 | 6.70 | 53.12 | 53.34 | 184.4 | |
| Pentanal | 51.87 | 51.79 | 8.87 | 8.69 | 39.68 | 39.51 | 241.0 | |
| Octanal | 58.94 | 58.76 | 10.09 | 9.86 | 31.50 | 31.37 | 292.7 | |
| Hexadecanal | 69.11 | 68.26 | 11.42 | 11.45 | 20.02 | 20.25 | | 39 |
| Ethylenethioketal of | | | | | | | | |
| 2-propanone | 45.27 | 45.39 | 7.76 | 7.51 | 47.91 | 47.76 | 178.5 | |
| 2-pentanone | 51.83 | 51.79 | 8.63 | 8.69 | 39.43 | 39.51 | 221.0 | |
| 2-octanone | 59.02 | 58.76 | 9.93 | 9.86 | 31.60 | 31.37 | 277.1 | |
| 2-nonadecanone | 70.55 | 70.32 | 11.61 | 11.80 | 17.76 | 17.87 | | 41 |

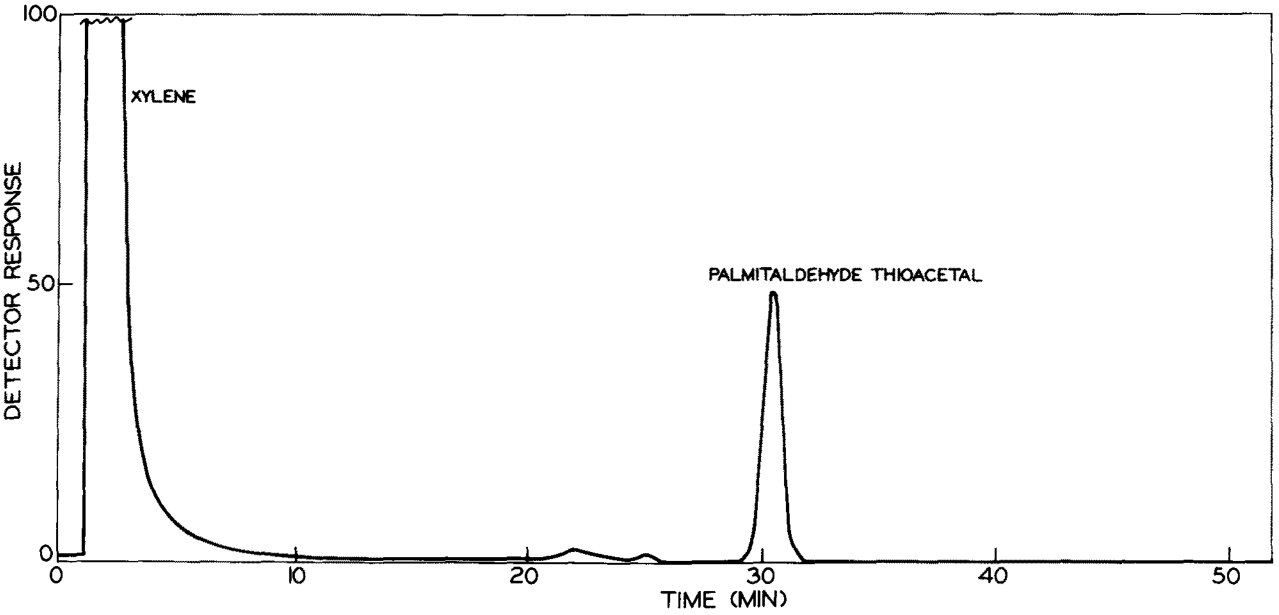


FIG. 3. A chromatogram showing the effect of a CaSO$_4$ column on eliminating the impurity peaks in the reaction of hexadecanal 2,4-DNPH.

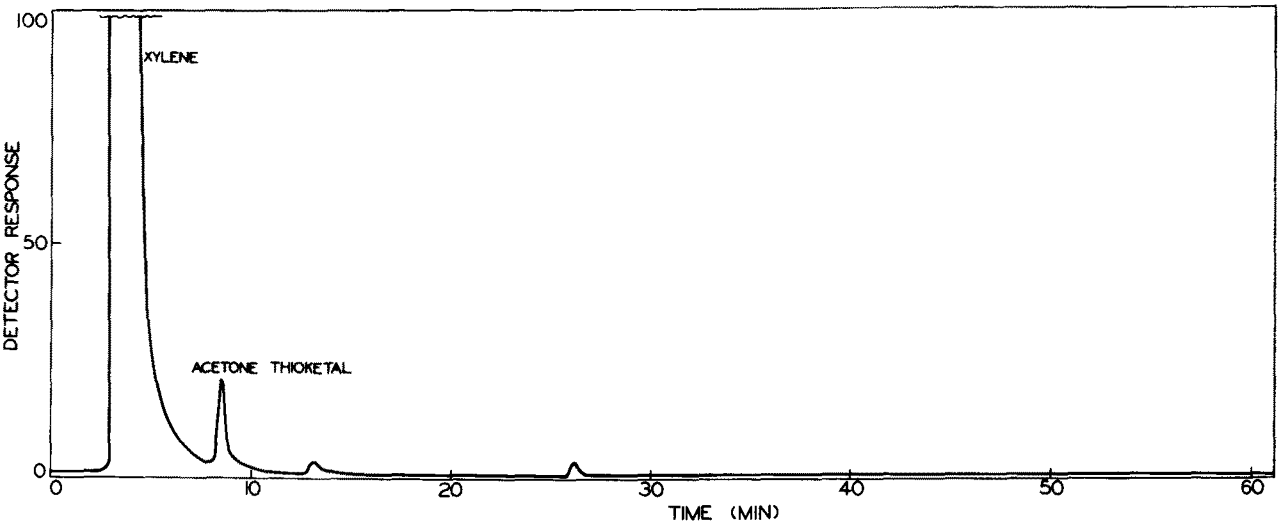


FIG. 4. A chromatogram of the reaction mixture of 2-propanone 2,4-DNPH using p-TSA catalyst.

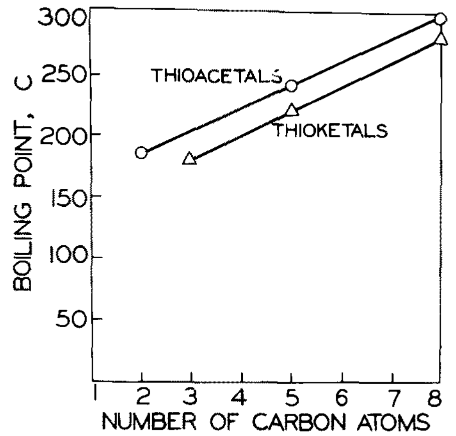


FIG. 5. The relationship of the boiling points of ethylenethioacetal and ethylenethioketal standards and the carbon number of their parent carbonyls.

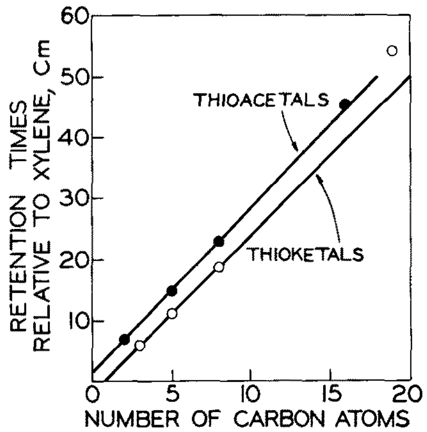


FIG. 6. The retention times of ethylenethioacetal and ethylenethioketal standards on an FFAP column in relation to the carbon number of the parent carbonyls.

gas and the hydrogen flow rates were 30 ml/min. Five foot by 1/8 in. stainless steel columns packed with 10% FFAP on 80/100 mesh DMCS-treated Chromosorb W were the major columns used throughout this study. Columns packed with 5% SE-30 on 60/80 mesh DMCS-treated Chromosorb W were also tried.

Injector temperatures were between 200-210 C and the detectors were at 240 C. Linear temperature programming of the columns was used. The sample was injected at a column temperature of 50 C. The temperature was programmed at the rate of 4 C/min until 215 C was reached and the temperature became isothermal. Retention times from the *o*-xylene solvent peak were used for peak identification.

### Thin Layer Chromatography

Thin layers of silica gel G were used and a Skelly Solve B-diethyl ether-acetic acid (90:10:1) solvent system was used for developing.

## EXPERIMENTAL PROCEDURES

### Conversion of the 2,4-Dinitrophenylhydrazones Into the Corresponding Ethylenethioacetal and Ethylenethioketal Using Boron Fluoride Ethyl Ether Catalyst

All reactants and empty tubes were flushed with nitrogen gas before use. The reaction was carried out in 50 or 100 ml glass high pressure tubes obtained from Fischer-Porter Co. A specific weight of the 2,4-DNPHS was dissolved in a minimum amount of chloroform. A volume of 1,2-ethanedithiol equivalent to 3.7 times the moles of $BF_3$ ethyl ether was added. The catalyst was added at a level of 80 times the moles of the derivative. After being flushed with nitrogen gas, the tube was closed tightly and heated at 125 C for 120 min. Subsequently, the tube was cooled in ice, and alkali (7.5 N NaOH in 50% methyl alcohol) was added, followed by pentane. The tube was centrifuged for 5 min, and the pentane layer was removed. The reaction mixture was extracted with pentane three more times. The pentane extract could be rewashed with alkali and dried over calcium sulfate, though it was usually unnecessary. Pentane was evaporated, and the residue was made to 5 ml with *o*-xylene and portions injected into the GLC.

### Calcium Sulfate Column

Removal of the interfering peaks which appeared in the chromatogram of the above extract was achieved with a calcium sulfate column. The $CaSO_4$ $2H_2O$ was washed with diethyl ether and then dried at 130 C for 18 hr. Silica Gel G was washed with diethyl ether and activated at 130 C for 48 hr. The column was prepared under hexane, and a nitrogen gas pressure of 10 lb was used for packing. Ten grams of Silica Gel G were packed at the bottom of an appropriate chromatographic glass column. In this investigation, a glass column, 2.5 cm, i.d., 30 cm long and with a 1 liter reservoir at the top, was used. Forty grams of activated $CaSO_4$ were packed on top of the silica gel. The pentane extract of the reaction was either evaporated and the residue was dissolved in a small volume of pentane, or the extract without evaporation was added to the top of the column. The column was then developed with 200 ml of pentane-ether (100:1-1/2). The column effluent was evaporated, and the residue was made to 5 ml with *o*-xylene and portions injected into the GLC.

### Conversion of the 2,4-Dinitrophenylhydrazones Into the Corresponding Ethylenethioacetal and Ethylenethioketal Using p-Toluenesulfonic Acid Catalyst

Twenty milligrams of the derivative were dissolved in 2 ml, 1,2-ethanedithiol, and 0.3 g p-TSA and 0.075 ml $H_2O$ were added. The tube was flushed with nitrogen gas, closed tightly, and heated at 200 C for 15 min. The tube was cooled in ice and 15 ml 7.5 N NaOH in 50% methyl alcohol were added. The reaction mixture was then extracted four times with pentane. The solvent was evaporated and the product was made to 5 ml with *o*-xylene and portions injected into the GLC.

## RESULTS

### Yields of the Ethylenethioacetals and Ethylenethioketals Obtained From 2,4-Dinitrophenylhydrazones of Various n-Alkanals and Methyl Ketones-Using Boron Fluoride Ethyl Ether Catalyst

As shown in Table I, yields obtained from long chain 2,4-DNPHS of $C_8$ and above, whether ketones or aldehydes, were over 90%. The same high yields were obtained from octanal and 2-nonadecanone. The short chain 2,4-DNPHS of $C_3$ and lower did not produce comparable high yields. 2-Propanone 2,4-DNPHS gave 85% and ethanal 2,4-DNPHS gave 75.5%. The high yields of the long chain derivatives did not require the severe treatment of 125 C for 120 min or the high levels of reactants. The yields in Table I were determined without the use of a $CaSO_4$ column since there were no side reaction peaks interfering with the products.

### Side-reactions

When $BF_3$ ethyl ether catalyst was used, side products were formed which were extracted and appeared as peaks in the chromatogram. As indicated in Figure 1, five major impurity peaks were obtained from the reaction of 2-propanone, 2,4-DNPH. Figure 2 (B) shows the reaction extract chromatographed on a thin layer of Silica Gel G. In the extract of the reaction of p-TSA (Fig. 2A) and the ethylenethioacetal and ethylenethioketal standards, most of the impurities moved behind the standards.

Figure 3 shows the effect of the $CaSO_4$ column on the GLC chromatogram of hexadecanal thioacetal. The column was effective in eliminating most of the impurities and the recovery of the product was complete.

### Yields of the Ethylenethioacetals and Ethylenethioketals Obtained From 2,4-Dinitrophenylhydrazones of Various n-Alkanals and Methyl Ketones Using p-Toluenesulfonic Acid Catalyst

As indicated in Table II, yields obtained from octanal-hexadecanal- and 2-octanone-2,4-DNPHS were determined on FFAP and SE-30 columns. Yields on both columns were essentially the same. The short chain 2,4-DNPH in each class gave the lowest yields, 78% and 72% for ethanal and 2-propanone, respectively. The highest yield, 93%, was obtained from 2-octanone 2,4-DNPH. Figure 4 shows the chromatogram of the 2-propanone 2,4-DNPH reaction mixture extract.

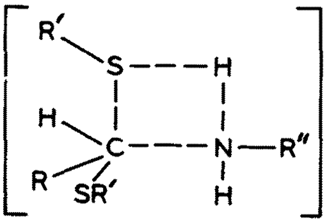
### Properties of Ethylenethioacetal and Ethylenethioketal Standards

Table III shows the observed elemental analysis compared to the theoretical values, the boiling points corrected to 760 mm pressure, and the melting points of eight standards. Figure 2 shows the thin layer chromatography (TLC) plate of these standards. Virtually all were characterized by one spot, although ethanol thioacetal showed an additional small spot close to the origin. The impurities, as determined by GLC, were 0% to 0.5% for all standards except for ethanal thioacetal, which was 1.4%. The elemental analysis of the standards were close to the theoretical values. Figure 5 indicates a linear relationship when the boiling points of the standards at 760 mm pressure were plotted against the carbon number of the parent carbonyls.

Figure 6 shows the relationship of the retention times on FFAP as measured from the xylene peak to the carbon number of the parent carbonyl of the standards. Each class yielded a linear relationship; however, the $C_{16}$ and $C_{19}$ peaks appeared in the isothermal period and, therefore, their retention times did not fall on the line. Figure 7 shows that the same relationship holds for the SE-30 column; however, the retention times of both classes were shorter than on FFAP. On both columns, thioketals had shorter retention times than the corresponding thioacetals.

## DISCUSSION

Thiols add to Schiff-bases to an appreciable extent in solution (13). The equilibrium constant of the addition of thiol to *n*-benzylideneaniline increased by the presence of an electron-withdrawing group on the *para*-position of the ring. This factor also caused the cleavage of the addition product to produce thioacetal, and catalyst was not needed. A yield of 52% thioacetal was obtained after 15 hr of reflux. The intermediate when monothiol was used was assumed to be the following.



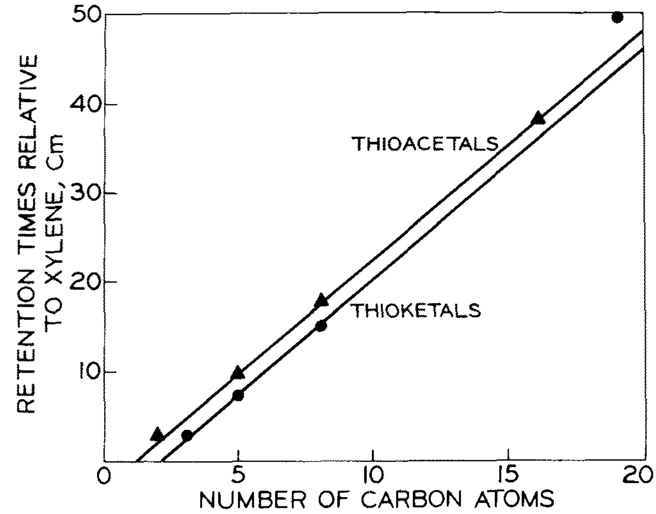
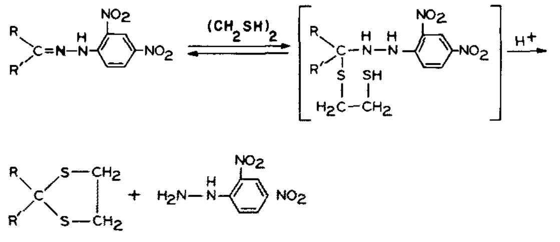


FIG. 7. The retention times of ethylenethioacetal and ethylenethioketal standards on a SE-30 column in relation to the carbon number of the parent carbonyls.

The 2,4-DNPHS are Schiff-bases that have two electron-withdrawing groups, and were found in this investigation to behave similarly to *n*-benzylideneaniline in their reaction with thiols. A dithiol was used since it was expected to facilitate the cleavage of the addition product and to increase the yields of ethylenethioacetal and ethylenethioketal as compared to a monothiol.

The use of an acid catalyst was essential, contrary to the findings of Oakes (13). For example, 2-propanone 2,4-DNPH did not product the ethylenethioketal when acid was omitted and heated to 200 C for 15 min.

The reactions in our case were assumed to go as follows.



The presence of the two nitro groups in the hydrazones, the use of the dithiol and the presence of acid catalyst helped the reaction so that it proceeded at temperatures as low as 40 C. This was particularly true for the long chain 2,4-DNPHS. Hexadecanal 2,4-DNPH, for example, gave quantitative conversion by heating at 130 C for 10 min using $BF_3$ ethyl ether catalyst. Short chain 2,4-DNPHS needed more drastic conditions to produce reasonable yields. High heat treatment of 125 C for 120 min was necessary for 2-propanone 2,4-DNPH crystals (melted at 127 C). In general, the reaction using p-TSA catalyst gave somewhat lower yields but far cleaner chromatograms than the reaction using $BF_3$ ethyl ether. The low yields for the short chain 2,4-DNPHS were not improved by further manipulation of reaction conditions.

The interfering peaks appearing in the chromatograms were of a monosulfide nature and were not affected by disulfide reducing agents. However, these peaks were eliminated by the $CaSO_4$ column. Moreover, the reaction using $BF_3$ ethyl ether catalyst produced gas that caused the pressure inside the tube to rise and, therefore, the use of a high pressure tube was essential. For these reasons, the use of the p-TSA was advantageous.

The use of GLC for ethylenethioacetals and ethylenethioketals separations and determinations was successful.

The derivatives appeared stable on both liquid phases. The homologous series in each class were well separated with reasonably long retention times and produced well-shaped peaks.

As compared to the regular dimethylacetals, this method had certain advantages. The derivative was formed for short and long chains of aldehydes and ketones. There was a high yield of conversion, and moreover, the derivatives were more stable on GLC columns.

## ACKNOWLEDGMENT

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## REFERENCES

1. Soukup, R.J., R.J. Scarpellino and E. Danielczik, Anal. Chem. 36:2255 (1964).
2. Fracchia, M.F., F.J. Schuette and P.K. Mueller, Environ, Sci. Technol. 1:915 (1967).
3. Galetto, W.G., R.E. Kepner and A.D. Webb, Anal. Chem. 38:34 (1966).
4. Leonard, R.E., and J.E. Kiefer, J. Gas Chrom. 4:142 (1966).
5. Shimizu, Y., S. Matsuto, Y. Ito and I. Okada, Jap. J. Agric. Chem. 43:211 (1969).
6. Mahadevan, V., P. Phillips and W.O. Lundberg, J. Lipid Res. 6:434 (1965).
7. Roa, R.V., S. Ramachandran and D.G. Cornwell, Ibid. 8:380 (1967).
8. Stein, R.A., and V. Slawson, J. Chromatogr. 25:204 (1966).
9. Stacy, G.W., R.I. Day and R.J. Morath, J. Amer. Chem. Soc. 80:3475 (1958).
10. Shriner, R.L., R.C. Fuson and D.Y. Curtin, "The Systematic Identification of Organic Compounds–A Laboratory Manual," Fifth Edition, John Wiley and Sons, New York, 1964.
11. Fieser, L.F., J. Amer. Chem. Soc. 76:1945 (1954).
12. Metwally, M.M.E., C.H. Amundson, and T. Richardson, Anal. Chem. 39:551 (1967).
13. Oakes, T.R., Ph.D. Thesis, Washington State University 1963; Diss. Abstr. 25:1552 (1964).

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